



Studies on *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* Disease Complex of *Lycopersicon esculentum* and its Management Through Integrated Approach

**ABSTRACT
THESIS**

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ABSTRACT

Tomato (*Lycopersicon esculentum*) is one of the most important crops and outranks all other vegetables except the potato crop in popularity and value in the world. This crop has been suffered with plant parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* species) and soil borne fungi, especially *Fusarium* and *Rhizoctonia* spp. resulting in heavy yield loss. Keeping in view the importance and ever increasing demand of tomato and the damaging potential of root-knot nematodes alone and in combination with several soil borne fungi, this study was carried out to generate information pertaining to the survey of tomato growing areas in western Uttar Pradesh for the distribution and severity of diseases caused by plant-parasitic nematodes and soil borne fungi, particularly regarding the singular and combined effect of *M. incognita*, *F. oxysporum* and *R. solani* on the growth and fruit yield of tomato cv. K-25, a favorite variety of farmers and consumers in this region. The effects were also made to develop an eco-friendly, economically and sustainable package of Integrated Disease Management practices to offset the losses caused by the disease complexes.

In order to assess the distribution of the plant parasitic nematode-fungal disease complexes of tomato, an extensive survey was conducted during the month of April, 2004 and March, 2005 in farmers' fields growing tomato in Agra, Aligarh, Bulandshahar and Mathura districts of Uttar Pradesh. Symptomalogical studies indicated the patchy appearance of the disease which included yellowing of leaves, chlorosis and wilting of the plants, which was commonly noticed in all the localities surveyed. Collar rot with wire stem was also observed with wilting at nursery and early stages of crop growth in most of the localities. However, occasionally low to severe galling was also observed in roots of affected plants. Field samples studied in the laboratory more often revealed the presence of *F. oxysporum* and *R. solani*. However, *F. solani*, *F. chlamydosporium*, *M. phaseolina*, *P. ultimum* and *P. aphanidermatum* were also isolated from such infected plants. The highest percent root infection by fungi was found in Agra followed by Aligarh, Bulandshahar and Mathura district, respectively.

Besides soil borne fungi, several important plant parasitic nematodes such as larvae of *Meloidogyne*, *Tylenchorhynchus*, *Hoplolaimus*, *Helicotylenchus*, *Rotylenchulus*

and *Xiphinema* spp. were also isolated from the rhizospheric soil of diseased plants of tomato grown in these localities. However, the population of *Pratylenchus*, *Tylenchus*, *Heterodera*, *Longidorus* and *Criconimoides* spp. were also occasionally yielded in isolations. Studies regarding the identification of species of root-knot nematodes from the galled roots indicated the presence of *M. incognita* singly in most of the localities surveyed. However, mixed infestation of *M. incognita* and *M. javanica* was also encountered from the same root system of infected plants collected during the survey. The highest population of larvae of *Meloidogyne* spp. in soil and also root-knot index was found in Mathura followed by Aligarh, Bulandshahar and Agra district, respectively.

During the survey attempts were also made to identify the potential fungal and bacterial biocontrol agents, particularly *T. harzianum*, *T. virens* and *P. fluorescens* from the farmers' fields growing tomato. Different isolates of potential biocontrol agents i.e. *T. harzianum* (12 numbers), *T. virens* (6 numbers) and *P. fluorescens* (17 numbers) were isolated and identified from rhizosphere soil of healthy plants collected from sick fields from different localities.

Pathogenicity tests conducted in green house on tomato cv. K 25, revealed the pathogenic potential of *M. incognita* and caused significant reduction in the number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight at all initial inoculum levels. In general, there was a positive relationship between the initial inoculum levels of *M. incognita* and reduction in all the test parameters. There was a negative relationship between initial inoculum densities and rate of nematode multiplication. Maximum root-knot index (4.00) and lowest nematode multiplication rate Rf (4.9) were observed at the highest Pi (25,000 J₂/5 kg soil), whereas, highest Rf (15.3) was observed at minimum Pi (2,500 J₂/5 kg soil).

Effect of different inoculum levels of *F. oxysporum* on disease development and on growth and fruit yield of tomato cv. K-25, exhibited a gradual increase in the reduction of number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight of tomato, and these increasing levels also increased percent root infection by the fungus. The maximum reduction in the corresponding test parameters was 39.6, 50.0, 34.7, 36.2, 37.1, 35.5, 40.3 and 38.3%, respectively at the highest initial inoculum level of 1.5×10^7 cfu/5 kg soil as compared to uninoculated control. At the

lowest Pi (2.5×10^6 cfu/5 kg soil), the root infection was 4.5% and at the highest Pi (1.5×10^7 cfu/5 kg soil), it was 50.5%.

Different initial inoculum levels of *R. solani* also exhibited its pathogenic potential on tomato cv. K-25 in terms of a gradual increase in the extent of reduction in number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight and also an increase in the percent root infection by the fungus. The maximum reduction in the corresponding test parameters was 53.8, 60.8, 45.2, 40.0, 42.1, 41.4, 45.6 and 45.5%, respectively at the highest initial inoculum level of 15.0 g mycelium/5 kg soil as compared to uninoculated control. At the lowest Pi (2.5 g mycelium/5 kg soil), root infection was 5.0% and at the highest Pi (15 g mycelium/5 kg soil), it was 65.0%.

Studies pertaining to the sequential, simultaneous and single inoculation of *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5×10^6 cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) on tomato cv. K-25 indicated that the highest reduction in fruit yield (83.3%), shoot dry weight (78.0%) and root dry weight (81.3%) was found in plants inoculated simultaneously with *M. incognita*, *F. oxysporum* and *R. solani* followed by *M. incognita* seven days prior to *F. oxysporum* and *R. solani*, *F. oxysporum* and *R. solani* seven days prior to *M. incognita*, *M. incognita* and *R. solani* simultaneously, *M. incognita* seven days prior to *R. solani*, *M. incognita* and *F. oxysporum* simultaneously, *M. incognita* seven days prior to *F. oxysporum*, *F. oxysporum* and *R. solani* simultaneously, *R. solani* seven days prior to *M. incognita*, *F. oxysporum* seven days prior to *M. incognita*, *M. incognita* alone, *R. solani* alone and *F. oxysporum* alone, respectively. However, the highest reproduction rate (11.8) of *M. incognita* and root-knot index (2.90) were observed in plants inoculated with the nematode alone, whereas, highest root infection (80.0%) by fungal pathogens was observed in plants inoculated with *M. incognita*, *F. oxysporum* and *R. solani* simultaneously.

In vitro screening of twelve isolates of *T. harzianum*, six isolates of *T. virens* and seventeen isolates of *P. fluorescens* against *F. oxysporum* and *R. solani*, using dual culture method, indicated that isolate TH-H-3 of *T. harzianum*, isolate TV-K-3 of *T. virens* and isolate PS-4 of *P. fluorescens* were the most promising as they significantly ($p \leq 0.05$) inhibited the mycelial growth of both the test pathogens. However, the bioefficacy of biocontrol agents varied with the pathogens. Similarly, the efficacy of

organic amendments (neem seed powder and farmyard manure) and pesticides (carbofuran, topsin-M and bavistin) was evaluated *in vitro* against *F. oxysporum* and *R. solani*. Among all the selected biocontrol agents, organic amendments and pesticides, cent percent inhibition in the mycelial growth of both the pathogens was observed in treatments with bavistin and topsin-M followed by *T. harzianum* isolate TH-H-3 (85.2, 100), *T. virens* isolate TV-K-3 (86.4, 92.2), *P. fluorescens* isolate PS-4 (65.9, 73.3), neem seed powder (21.1, 13.3), carbofuran (11.1, 3.3) and farmyard manure (6.7, 1.1), respectively.

Comparative efficacy of all treatments including promising isolates of biocontrol agents viz., *T. harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 and *P. fluorescens* isolate PS-4 (50 mg/kg soil each), organic amendments viz., neem seed powder (250 mg/kg soil) and farmyard manure (1500 mg/kg soil) and pesticides viz., carbofuran (33.4 mg/kg soil), topsin-M (1.4 mg/kg soil) and bavistin (2 mg/kg soil) was studied in pot conditions against the management of disease complex of *M. incognita* alone, *F. oxysporum* alone, *R. solani* alone, *F. oxysporum* + *R. solani* simultaneously, *M. incognita* + *F. oxysporum* simultaneously, *M. incognita* + *R. solani* simultaneously and *M. incognita* + *F. oxysporum* + *R. solani* simultaneously infesting tomato cv. K-25.

Among the various treatments, carbofuran was found highly effective against *M. incognita* in increasing the plant growth and fruit yield and reducing the root-knot index followed by neem seed powder, *T. harzianum*, *P. fluorescence*, *T. virens*, bavistin, topsin-M and farmyard manure, as compared to untreated inoculated plants.

However, in pots inoculated with *F. oxysporum* alone, *T. harzianum* gave maximum increase in plant growth and fruit yield and reduced the percent root infection. Other effective treatments in descending order were bavistin, *P. fluorescence*, topsin-M, *T. virens*, neem seed powder, carbofuran and farmyard manure.

The highest increase in plant dry weight and fruit yield and lowest root infection by *R. solani*, was also found in plants treated with *T. harzianum* followed by topsin-M, *P. fluorescence*, bavistin, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively.

In plants inoculated with *F. oxysporum* and *R. solani* simultaneously, the treatment with *T. harzianum* was found highly effective in increasing plant dry weight

and fruit yield and in decreasing root infection by both the pathogens followed by bavistin, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, carbofuran and farmyard manure, respectively as compared to untreated inoculated plants.

In simultaneously inoculated plants with *M. incognita* + *F. oxysporum*, *T. harzianum* appeared to be highly effective in increasing plant dry weight and fruit yield and in decreasing root infection by the fungus, followed by carbofuran, *P. fluorescens*, bavistin, *T. virens*, neem seed powder, topsin-M and farmyard manure, respectively as compared to untreated inoculated plants.

Highest plant dry weight and fruit yield and lowest root-knot index and root infection due to combined inoculation of *M. incognita* and *R. solani* was found in plants treated with *T. harzianum* followed by carbofuran, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, bavistin and farmyard manure, respectively as compared to untreated inoculated plants.

In mixed inocula of *M. incognita*, *F. oxysporum* and *R. solani*, the most satisfactory results were obtained with the treatment of *T. harzianum* followed by *P. fluorescens*, *T. virens*, carbofuran, bavistin, neem seed powder, topsin-M and farmyard manure, respectively. These treatments were found to improve plant growth and fruit yield of tomato cv. K25, thereby decreased root-knot index and percent root infection.

The compatibility test of bioinoculants and there of with organic amendments and pesticides was studied *in-vitro* and the observations indicated that *T. harzianum* isolate TH-H-3 and *T. virens* isolate TV-K-3 were found to be 100 % and 77.8 % compatible, respectively with *P. fluorescens* isolate PS-4. However, in the case of organic additives, all the biocontrol agents were 100 % compatible with neem seed powder and farmyard manure. In case of pesticides, all the biocontrol agents were found to be 100 % compatible with carbofuran. *T. harzianum* and *T. virens* and 100 % incompatible with topsin-M and bavistin. While, *P. fluorescence* was found 100 % compatible with both the fungicides.

The compatible and effective treatment components viz., bioinoculants, pesticides and organic amendments were also evaluated for their efficacy against *M. incognita*, *F. oxysporum*, *R. solani*, using alone and in combinations under pot conditions. Various treatments applied were *T. harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 and *P.*

fluorescens isolate PS-4 @ 100 kg/ha, organic amendments viz., neem seed powder @ 500 kg/ha and farmyard manure @ 3,000 kg/ha and pesticides viz., carbofuran, topsin-M, bavistin @ 2 kg a.i./ha each alone and in combinations [rate of application was reduced to half (for integration of two additives), one third (for integration of three additives) and one fourth (for integration of four additives) of the standard rate] under nursery beds in sick field conditions. Results indicated that all treatments applied singly or in combinations were able to increase the number of emerging seedlings and their fresh weight coupled with considerable reduction in disease incidence. Among all the treatments applied singly, bavistin was found most effective in increasing the emergence of number and fresh weight of seedlings and reducing the disease incidence. When one half dose was applied in the combined treatments of two additives, *P. fluorescens* + bavistin was found the best treatment in increasing the number of emerging seedlings and their fresh weight and in reducing the disease incidence. In case of integrated treatment with three additives applied as one third dose of the standard doses, the treatment with *T. harzianum* + neem seed powder + carbofuran proved best in increasing the number of emerging seedlings and their fresh weight and in reducing disease incidence. Among all integrated treatments with four additives applied as one fourth dose of the standard dose, the *T. harzianum* + farmyard manure + neem seed powder + carbofuran was found highly effective in increasing germination (185.6%) and fresh weight of seedlings (262.7%) and in reducing disease incidence [RKI (0.0) and PRI (7.5)].

The effective treatments or their combinations were then tested under sick field conditions for the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25. The results indicated that all component of treatments used alone or combined irrespective of doses, considerably increased plant growth and fruit yield and reduced disease intensity as compared to untreated control. *T. harzianum* + farmyard manure + neem seed powder + carbofuran (all the additives applied as one fourth doses of standard dose) was found most effective in increasing plant growth and in reducing disease intensity followed by *P. fluorescens* + farmyard manure + neem seed powder + carbofuran (one fourth doses), *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses), *T. harzianum* + neem seed powder +

carbofuran (one third doses), *T. virens* + farmyard manure + neem seed powder + carbofuran (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + topsin-M (one fourth doses), *P. fluorescens* + neem seed powder + carbofuran (one third doses), *P. fluorescens* + neem seed powder + bavistin (one third doses), *T. virens* + neem seed powder + carbofuran (one third doses), *T. harzianum* + *P. fluorescens* + neem seed powder (one third doses), *T. virens* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + neem seed powder + topsin-M (one third doses) and *T. harzianum* + farmyard manure + carbofuran (one third doses), respectively.

The studies have, thus, revealed that biocontrol agents and organic amendments are the most important component package of integrated management system as they have proved effective for reducing the infectivity of *M. incognita*, *F. oxysporum* and *R. solani* disease complex of tomato. In these studies, the treatments have been evaluated under pot, nursery and sick field conditions, wherein high population densities of the pathogens were present. In the farmers' fields, the inoculum levels may vary from low to high, but even in high inoculum densities, the integrated treatments proved a better option for the management of disease complex, and thus, enhancing yield.

The studies have generated knowledge on the damage caused by *M. incognita* - *F. oxysporum* - *R. solani* disease complex of tomato and the development of effective integrated management options. This information is not only of academic importance, but it also has usefulness for tomato growers in increasing the productivity per unit area. It is concluded that farmers should have prior information of their field/soil infestation level with these pathogens and if needed may adopt proper management options to save the crop from this disease complex.

On the basis of the above studies carried out for Ph. D. work, application of *T. harzianum* or *P. fluorescens* (25 kg/ha) + farmyard manure (750 kg/ha) + neem seed powder (125 kg/ha) + carbofuran (16.5 kg/ha) may be recommended to get the best results for the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex in tomato under large scale cultivation.



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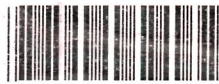
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*To
My
Beloved
Parents*



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Certificate

This is to certify that the work embodied in this thesis entitled “*Studies on Meloidogyne incognita, Fusarium oxysporum and Rhizoctonia solani disease complex of Lycopersicon esculentum and its management through integrated approach*” submitted for the degree of Doctorate of Philosophy in Agriculture (Plant Protection) carried out by **Mr. Vipin Kumar** under my supervision, is the original research work. This work has not been submitted either partially or fully to this or other University/Institute for the award of any other degree/diploma. The candidate has fulfilled the prescribed conditions given in the ordinance and regulations of Aligarh Muslim University, Aligarh (U.P.), India.


16.09.2008
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Supervisor

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| 2= 2.5 g mycelium/5 kg soil | 6= 12.5 g mycelium/5 kg soil |
| 3= 5.0 g mycelium/5 kg soil | 7= 15.0 g mycelium/5 kg soil |
| 4= 7.5 g mycelium/5 kg soil | |

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| 3= 7.5x10 ⁶ cfu of <i>F. o.</i> /5 kg soil | 7= <i>M. i.</i> + <i>R. s.</i> /5 kg soil |
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- | | |
|---|--|
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| 8= <i>M. i.</i> + <i>F. o.</i> + <i>R. s.</i> /5kg soil | 12= <i>F. o.</i> (pre)+ <i>M. i.</i> (post)/5 kg soil |
| 9= <i>M. i.</i> (pre)+ <i>F. o.</i> (post)/5kg soil | 13= <i>R. s.</i> (pre)+ <i>M. i.</i> (post)/5kg soil |
| 10= <i>M. i.</i> (pre)+ <i>R. s.</i> (post)/5kg soil | 14= <i>F. o.</i> + <i>R. s.</i> (pre)+ <i>M. i.</i> (post)/5kg soil |

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| 3= TH-AG-5 | 9= TH-K-9 | 3= TV-M-1 |
| 4= TH-MN-2 | 10= TH-BS-6 | 4= TV-AG-3 |
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| 2= TH-M-7 | 8= TH-AL | 2= TV-H |
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|----------------|---------|--------------|--------|

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2= AG-4	7= PS-7	3= NK-1	8= M-3
3= RGP-1	8= PS-9	4= NK-2	9= M-5
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1= AG-1	6= PS-4	2= UIP-2	7= K-3
2= AG-4	7= PS-7	3= NK-1	8= M-3
3= RGP-1	8= PS-9	4= NK-2	9= M-5
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3= Neem seed powder	6= Bavistin

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2= Inoculated untreated	5= <i>P. fluorescens</i> @ 250 mg/5 kg soil
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7= NSP @ 1250 mg/5 kg soil	10= Bavistin @ 10 mg/5 kg soil

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2= Inoculated untreated	5= <i>P. fluorescens</i> @ 250 mg/5 kg soil
3= <i>T. harzianum</i> @ 250 mg/5 kg soil	6= FYM @ 7500 mg/5 kg soil

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|----------------------------|----------------------------------|
| 1= Uninoculated untreated | 8= Carbofuran @ 167 mg/5 kg soil |
| 2= Inoculated untreated | 9= Topsin-M @ 12 mg/5 kg soil |
| 7= NSP @ 1250 mg/5 kg soil | 10= Bavistin @ 10 mg/5 kg soil |

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|---|---|
| 1= Uninoculated untreated | 4= <i>T. virens</i> @ 250 mg/5 kg soil |
| 2= Inoculated untreated | 5= <i>P. fluorescens</i> @ 250 mg/5 kg soil |
| 3= <i>T. harzianum</i> @ 250 mg/5 kg soil | 6= FYM @ 7500 mg/5 kg soil |

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- | | |
|----------------------------|----------------------------------|
| 1= Uninoculated untreated | 8= Carbofuran @ 167 mg/5 kg soil |
| 2= Inoculated untreated | 9= Topsin-M @ 12 mg/5 kg soil |
| 7= NSP @ 1250 mg/5 kg soil | 10= Bavistin @ 10 mg/5 kg soil |

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- | | |
|---|---|
| 1= Uninoculated untreated | 4= <i>T. virens</i> @ 250 mg/5 kg soil |
| 2= Inoculated untreated | 5= <i>P. fluorescens</i> @ 250 mg/5 kg soil |
| 3= <i>T. harzianum</i> @ 250 mg/5 kg soil | 6= FYM @ 7500 mg/5 kg soil |

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- | | |
|----------------------------|----------------------------------|
| 1= Uninoculated untreated | 8= Carbofuran @ 167 mg/5 kg soil |
| 2= Inoculated untreated | 9= Topsin-M @ 12 mg/5 kg soil |
| 7= NSP @ 1250 mg/5 kg soil | 10= Bavistin @ 10 mg/5 kg soil |

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- | | |
|---|---|
| 1= Uninoculated untreated | 4= <i>T. virens</i> @ 250 mg/5 kg soil |
| 2= Inoculated untreated | 5= <i>P. fluorescens</i> @ 250 mg/5 kg soil |
| 3= <i>T. harzianum</i> @ 250 mg/5 kg soil | 6= FYM @ 7500 mg/5 kg soil |

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- 1= Uninoculated untreated 4= *T. virens* @ 250 mg/5 kg soil
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- 1= Uninoculated untreated 8= Carbofuran @ 167 mg/5 kg soil
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- 1= Uninoculated untreated 4= *T. virens* @ 250 mg/5 kg soil
2= Inoculated untreated 5= *P. fluorescens* @ 250 mg/5 kg soil
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- 1= Uninoculated untreated 8= Carbofuran @ 167 mg/5 kg soil
2= Inoculated untreated 9= Topsin-M @ 12 mg/5 kg soil
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pesticides

- | | |
|----------------------|---------------|
| 1= Untreated control | 4= Carbofuran |
| 2= Farmyard manure | 5= Topsin-M |
| 3= Neem seed powder | 6= Bavistin |

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- | | |
|----------------------|---------------|
| 1= Untreated control | 4= Carbofuran |
| 2= Farmyard manure | 5= Topsin-M |
| 3= Neem seed powder | 6= Bavistin |

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- | | |
|------------------------|------------------------|
| 1= Untreated control | 4= Th + Pf + FYM + NSP |
| 2= Th + FYM + NSP + Cf | 5= Pf + FYM + NSP + B |
| 3= Pf + FYM + NSP + Cf | |

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 (B) A view of best treatment combination with untreated control
 1= Untreated control 2= Treated with Th + FYM + NSP + Cf

ABBREVIATION USED

Abb.	
g	Gram
mg	Milligram
m	Meter
cv.	Cultivar
J ₂	Second stage juvenile
Pi	Initial population
kg	Kilogram
cfu	Colony forming unit
f. sp.	Formae speciales
AG	Anastomosis group
a.i.	Active ingredient
ha	Hectare
w/w	Weight/ weight
ml	Milli liter
q	Quintal
h	Hour
Min	minute
v/v	Volume/volume
t	Ton
PDA	Potato dextrose agar
PPM	Part per million
µg	Micro gram
µm	Micro meter
BOD	Biological oxygen demand
PDB	Potato dextrose broth
w/v	Weight/volume
cm	Centimeter
°C	Degree centigrade
G	Granule
WP	Wettable powder
FYM	Farm yard manure
NSP	Neem seed powder
MOC	Mustard oil cake
PR	Poultry reguse
RBD	Randomized Block Design
ANOVA	Analyses of variance
P	Probability level
CD	Critical difference
LSD	Least significant difference
sp.	Species
PRI	Percent root infection
RKI	Root-knot index
Rf	Reproduction factor

Pf	Final population
Fig.	Figure
MBC	Methyl-2-benzimidazole carbamate
EBC	Ethyle-2-benzimidazole carbamate
IPM	Integrated pest management
UK	United Kingdom
UP	Uttar Pradesh
DD	1, 3 Dichloro propene + 1, 2 Dichloropropane
EDB	Ethylene di bromide
l	liter
@	At the rate of
ABCD	Attapulgate based clay dust
MOC	Mustard oil cake
PR	Poultry refuse
PCNB	Penta chloro nitro benzene
USA	United States of America
FAO	Food and Agriculture Organization
KCl	Potassium chloride
KOH	Potassium hydroxide
mm	Milli meter
Min	Minute
Rf	Reproduction factor
<i>Trh</i>	<i>Tylenchorhynchus</i>
<i>Roty</i>	<i>Rotylenchulus</i>
<i>Hel</i>	<i>Helicotylenchus</i>
<i>Hop</i>	<i>Hoplolaimus</i>
<i>Het</i>	<i>Heterodera</i>
<i>Tyl</i>	<i>Tylenchus</i>
<i>As</i>	<i>Aspergillus</i>
<i>Al</i>	<i>Alternaria</i>
<i>Ph</i>	<i>Phoma</i>
<i>Ac</i>	<i>Acremonium</i>
<i>Peni</i>	<i>Penicillium</i>
<i>Cur</i>	<i>Curvularia</i>
<i>M i</i>	<i>Meloidogyne incognita</i>
<i>F o</i>	<i>Fusarium oxysporum</i>
<i>R s</i>	<i>Rhizoctonia solani</i>
<i>Th</i>	<i>Trichoderma herzianum</i>
<i>Tv</i>	<i>Trichoderma virens</i>
<i>Pf</i>	<i>Pseudomonas fluorescens</i>
Cf	Carbofuran
T-M	Topsin-M
Bv	Bavistin

CHAPTER – 1

INTRODUCTION

Vegetables are a vital source of minerals, vitamins and dietary fibres and thus play an important role in human nutrition in supplying adequate quantity of free radicals, antioxidants and micronutrients (Kalloo and Pandey, 2002). India is the second largest producer of vegetables next to China in the world accounting for about 10 percent of the world's production. In 2007, total vegetable production in India was 72.54 million tonnes from 5.67 million hectare of land (FAO, 2008).

Among vegetable crops, tomato (*Lycopersicon esculentum*, Family- Solanaceae) is one of the most important crops and today it outranks all other vegetables except potato in popularity and value throughout the world. Tomato contributes about 7.7% of the total production of vegetables in 2007 with the production of 8.58 million tonnes from 0.48 million hectare (FAO, 2008). It is the native of tropical America and Mexico. It is an annual crop and grows well in high organic matter containing loam and silty loam soils having a pH range between 6.0 and 8.0. However, hybrid tomato can well adapt to all agro-climatic conditions (Singh *et al.*, 2004 b).

The tomato fruits are eaten raw and cooked. Large quantities of tomato are used to produce soup, juice, ketchup, pickles, puree, paste and powder. A ripe tomato (100g) contains as much as 94.10 g water, 1.00 g protein, 0.30 g fat, 4.00 g carbohydrate, 0.60 g fibre, 1100 I.U. vitamin A, 0.20 mg vitamin B, 23.00 mg vitamin C, 0.27 mg vitamin E, 0.60 mg nicotinic acid, 0.31 mg pantothenic acid, 0.004 mg biotin, 150.00 mg malic acid, 390.00 mg citric acid, 3.50 mg oxalic acid, 3.00 mg sodium, 268.00 mg potassium, 0.10 mg copper, 11.00 mg magnesium, 0.60 mg iron, 0.19 mg manganese, 27.00 mg phosphorus, 11.00 mg sulphur, 51.00 mg chlorine (Chatfield, 1949, 1954). It also has many other uses. Tomato seeds contain 24% oil. The semidried oil is used as salad oil and in the manufacture of margarine. Tomato is also popular as it adds a variety of colour and flavour to food. Tomato is also used for medicinal purposes. The pulp and juice are digestible, mild aperients, promoter of gastric secretions and blood purifier. It is also considered to be intestinal antiseptic and useful in canker of the mouth, sore mouth etc. (Tiwari and Chaudhury, 1993).

Like many vegetable crops, tomato is also affected by several diseases caused by nematodes, fungi, bacteria and viruses, leading to severe yield losses (Berkley, 1925; Horsfall, 1930; Sasser, 1979, 1989; Jones *et al.*, 1991; Rosello *et al.*, 1996; Decoteau, 2000; Netscher and Sikora, 2002; Haseeb, 2003; Walker, 2004).

Nematodes, in general, possess elongate, cylindrical or worm-like body, and are usually unciliated. They are triploblastic, bilaterally symmetrical, unsegmented, and pseudocoelomate animals. They are not only a diverse group of animals but like bacteria, viruses and insects also occupy all biotopes. Despite their low evolutionary status, they are found in almost every kind of environment. They are highly diverse in habitats such as soils, fresh water and oceans ranging from Long's Peak of Colorado (over 14,000 feet) to the floor of the arctic and antarctic. They develop in unimaginable numbers (millions/m² soil) and in wide variety of shapes and sizes (Luc *et al.*, 2002). A single acre of soil from arable land may contain as many as 3×10^9 nematodes, while marine beach sand may contain 15×10^8 nematodes per acre. The estimate of existing species of nematodes is around 5×10^5 or more, mostly parasitizing vertebrates, invertebrates like molluscs, crustaceans, insects, centipedes, millipedes, annelids, free living aquatic / marine / fresh water, soil inhabiting and plant parasitic species (Jairajpuri, 1988).

Plant parasitic nematodes constitute only 10% of the total nematode species, yet a plant is often attacked by more than one species. Usually, they are found in and around the roots of their host plants or in the stems, leaves and seeds of host plants (Goodey *et al.*, 1965; Webster, 1972; Lamberti and Taylor, 1979; Luc *et al.*, 2002). They produce disease symptoms on susceptible hosts and on a variety of crops such as vegetable, ornamental, fibre, fruit, oilseed, plantation, medicinal, and aromatic plants (Webster, 1972; Haseeb, 1992, 1994, 2003; Luc *et al.*, 2002; Haseeb and Shukla, 2007).

On a worldwide basis, the ten most important genera of plant parasitic nematodes have been reported to be *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Ditylenchus*, *Globodera*, *Tylenchulus*, *Xiphinema*, *Radopholus*, *Rotylenchulus*, and *Helicotylenchus*. They are considered as the most important nematodes in terms of distribution, host range and resulting yield losses (Sasser, 1980, Sasser and Freckman, 1987). The estimated overall average annual yield loss of the world's major crops due to plant parasitic nematodes being 12.3%. Monetary losses due to nematode damage in 21 crops, 15 of

which are “life sustaining”, were estimated at US \$ 77 million annually based on 1984 production figures and prices (Sasser and Freckman, 1987).

Root-knot nematodes (*Meloidogyne* species) are considered to be the most potential pest of many crops due to their worldwide distribution, extensive host range and highly advanced mode of parasitism. They produce both above and below ground symptoms on the host crop (Webster, 1972; Luc *et al.*, 2002). The above ground symptoms of their attack is “patchy” appearance due to uneven distribution of the nematode. Generally, diseased plants show slight to severe stunting, chlorosis and wilting. The poorly growing patches increase in size each year and may spread more rapidly in the direction of ploughing and irrigation. The symptoms advance with the age of the crop and are more spectacular under drought or low fertility levels (Webster, 1972; Sasser, 1989; Luc *et al.*, 2002; Haseeb, 2003; Haseeb and Shukla, 2007).

The most distinctive symptoms are observed on underground parts, which include galls or knots on the roots or other underground plant parts. In case of multiple infections on the nearby tissues, galls may coalesce to form large galls, terminally or sub terminally on the infected roots and their size varies with the species of *Meloidogyne* and hosts (Webster, 1972; Sasser and Carter, 1985; Sasser, 1989; Trudgill and Blok, 2001; Luc *et al.*, 2002; Haseeb, 2003; Haseeb and Shukla, 2007).

The extent of damage caused by the root-knot nematodes is governed by a number of factors such as nematode species, size of nematode population, susceptibility of host plants, environmental factors and presence of other organisms (Wallace, 1963, 1969; Webster, 1972, 1985; Norton, 1978; Lamberti and Taylor, 1979; Van Gundy, 1985; Veech and Dickson, 1987; Haseeb, 1992, 1994, 2003; Haseeb *et al.*, 1996, 1998, 1999, 2000 a, b, 2004; Trudgill and Blok, 2001; Haseeb and Shukla, 2007).

Ninety seven species of *Meloidogyne* have been described (Vyas and Patel, 2002). Trudgill and Blok (2001) reported that *Meloidogyne* spp. cause root-knot disease in over 2,50,000 types of flowering plants. In India, about half a dozen species of root-knot nematodes cause severe damage to various crops, including pulses, vegetables, plantation crops and medicinal and aromatic plants (Sitaramaiah, 1984; Haseeb, 1992, 1994; Luc *et al.*, 2002; Gaur *et al.*, 2004; Haseeb and Shukla, 2007). In fact, they are responsible for 95% of total crop loss attributed to the nematodes (Dasgupta, 1997). Although the extent

of crop losses due to root-knot nematodes is difficult to quantify, several workers have estimated approximate losses (Good, 1973; Lamberti, 1979; Wittwer, 1981; Sasser and Freckman, 1987; Sasser, 1989; Haseeb, 1992, 1994, 2003, 2004; Haseeb and Shukla, 2007).

The economic losses caused by root-knot nematodes depend not only on the severity of the nematode attack but also on the cash value of the crop (Franklin, 1979). Good (1973) reported that root-knot nematodes are responsible for about 40% of the loss due to all plant parasitic nematodes in the USA, which is about 10% of the total loss caused by all organisms. Bhatti and Jain (1977) estimated 46% yield loss of tomato due to *M. incognita*. Lamberti (1979) reported that root-knot nematodes cause as high as 85% reduction in yield of tomato. Sasser (1979) estimated that losses due to *Meloidogyne* species to vegetable crops in the tropics ranged from 24 - 38% on tomato. Later, Sasser (1989) estimated the average yield loss 20.6% on tomato due to *Meloidogyne* species.

Out of many species of root-knot nematodes infesting tomato, *M. incognita* has been considered the most serious threat to the cultivation of different varieties of tomato (Franklin, 1979; Lamberti, 1979; Sasser, 1979, 1989; Daiber, 1989; Ehwaeti *et al.*, 1998, 2000; Haseeb, 2003).

Fungi are a very large and diverse group of organisms, which have a unique life style. They have worldwide distribution and successfully exploit many different habitats (Isaac, 1992). Fungi are of great economic importance to man and play an important role in the disintegration of organic matters. They affect us directly by destroying food, fabric, leather and other commercial goods. They are responsible for a large number of diseases of plants, animals and man (Mehrotra, 1993).

The ability of fungi to adapt to a wide range of conditions makes them an important group of pathogens. More than 10,000 species of fungi are responsible for various diseases in plants. In general, all plants are attacked by some kind of fungi and each of the parasitic fungi can attack one or many species of plants. Almost all pathogenic fungi spend part of their life on the host plants and part in the soil or in the plant debris on the soil (Agrios, 2005).

Diseases of economically important crops cause 13 to 20% annual loss in production, representing US \$ 50×10^9 (James, 1981). In the United States, with the most

advanced disease management technologies, the extent of crop losses is similar (Lewis and Papavizas, 1991). A total of 65 million tonnes pre harvest losses in vegetables due to disease alone in the world have been estimated as 10.5%. Maximum loss of 44 million tonnes is reported only from developing countries (Panday *et al.*, 2002). In the absence of exact estimates for India, it can be safely assumed that more than 50% of the crop loss is due to soil inhabiting microorganisms (Gokulapalan *et al.*, 2000; Sen, 2000). Ten fungal genera viz., *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Sclerotium*, *Phytophthora*, *Verticillium*, *Macrophomina*, *Aspergillus* and *Colletotrichum* have been recognized playing a major role in the root disease complex as they cause seed decay, damping off, root-, collar-, crown- and foot-rots, seedling blight and wilt (Baker, 1970; Cook and Baker, 1983; Weller *et al.*, 2002; Haseeb, 2003).

Various plant pathogenic fungi are known to infect tomato, causing severe damage to crop. Most species of fungi responsible for causing diseases of tomato belong to the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Pythium*, *Phytophthora*, etc. (Berkley, 1925; Horsfall, 1930; Papavizas, 1969; Baker, 1970; Dhingra *et al.*, 1976; Lewis, 1979; Beckman, 1987; Elias and Schneider, 1991; Haseeb, 2003; Agrios, 2005).

Fusarium (Family - Tuberculariaceae) is an imperfect fungus, which occurs as soil borne facultative parasite in nature. Major species of this fungus (*F. oxysporum*, *F. solani*, *F. roseum*, *F. tricinctum* and *F. nivale*) have been reported (Toussoun and Nelson, 1975; Nelson *et al.*, 1981) to cause wilt disease in many crops. Several other species cause seed rot, damping off and root rot of vegetables, fruits, ornamentals, fiber crops and forest trees (Nelson, 1981; Beckman, 1987; Hillocks and Waller, 1997; Sen, 2000; Zamankova and Labeda, 2001; Weller *et al.*, 2002). Some species of *Fusarium* are responsible for causing wilt disease such as *F. oxysporum* f. sp. *lycopersici* on tomato, *F. oxysporum* f. sp. *niveum* and *F. oxysporum* f. sp. *batatas* on potato, *F. oxysporum* f. sp. *cepae* on onion, *F. oxysporum* f. sp. *conglutinans* on cabbage, *F. oxysporum* f. sp. *cubense* on banana, *F. oxysporum* f. sp. *vasinfectum* on cotton, *F. oxysporum* f. sp. *dianthii* on carnation, *F. oxysporum* f. sp. *chrysanthemi* on chrysanthemum and *F. oxysporum* f. sp. *udum* on pigeonpea (Booth, 1971; Armstrong and Armstrong, 1975; Toussoun and Nelson, 1975; O'Donnell, 1996; Zemankova and Lebeda, 2001; Sharma, 2003; Haseeb, 2003).

Tomato is susceptible to more than 200 diseases resulting in 75-95% yield loss. Fusarium wilt caused by pathogenic *F. oxysporum* is the most devastating disease resulting in 10-50% losses around the world (Lukyanenko, 1991; Weller *et al.*, 2002). Plants infected with *Fusarium* show stunting, gradual wilting and finally death occurs. Initially, yellowing of older leaves occurs and gradually most leaves turn yellow and wilt. Roots become dry and vascular system turns brown. Occasionally, entire fields of tomatoes are killed or severely damaged before a crop can be harvested (Jones *et al.*, 1991; Hartman and Datnoff, 1997; Roberts *et al.*, 2001; Haseeb, 2003; Agrios, 2005).

Besides *Fusarium* species, *Rhizoctonia* is the next most important pathogen of tomato. *R. solani*, the most widely recognized species of *Rhizoctonia* was originally described by Julius Kuhn on the potato in 1858. This fungus has long been called as a sterile fungus, as it was thought to be incapable of producing any type of spore. But now several species of *Rhizoctonia* have been reported to reproduce asexually as well as sexually. Thus, *R. solani* that produces sexual spores, the basidiospores, has now been put under the Family - Ceratobasidiaceae in the order - Tulasinallales. The sexual fruiting structures and basidiospores (i.e. teleomorph) were first observed and described in detail by Prillieux and Delacroiz in 1891. Its perfect stage has been named as '*Thanatophorus cucumeris*'.

R. solani is one of the most destructive species occurring globally and causing various maladies starting from seed decay, damping off, wire stem, root and stem rots, canker, sheath blight and ear rot on more than 500 hosts (Crosier, 1943; Baker, 1947, 1970; Sherf and Mac Nab, 1986; Ogoshi, 1985, 1996; Agrios, 2005). Its infection occurs on roots, tubers and other plant parts, which develop below or above ground (Hedgecock, 1904; Bewley, 1923; Roth and Ricker, 1943; Parmeter, 1970; Haseeb, 1983; Agrios, 2005). Very young seedlings may be killed before, or soon after, they emerge from the soil. After seedlings have emerged, the fungus attacks their stem and makes it water soaked, and incapable of supporting the seedlings, which then fall over and die. In older seedlings, the invasion of the fungus is limited to the outer cortical tissues, which develop elongate tan to reddish-brown lesions. The lesions may increase in length and width until they finally girdle the stem, and the plant may die. Before the plant dies the stem turns brownish black and may bend or become twisted without breaking, and this condition is

named as “wire stem” (Papavizas and Davey, 1961; Papavizas, 1969; Baker, 1970; Lewis, 1979; Haseeb, 1983; Sen, 2000; Agrios, 2005). The fungus causes pre-emergence decay of seeds and seedlings of tomato. The post-emergence mortality of seedlings at soil level is prominent in tomato and other bedding plants (Stephens *et al.*, 1982; Lumsden and Locke, 1989; Sokhi *et al.*, 1998; Haseeb, 2003).

In nature, plants are rarely exposed to the influence of a single pathogen. Fawcett (1931) recognized that nature does not work with pure culture and that associated organisms influence many plant diseases. Root grows in soil having a great number of microorganisms, whose action is often combined to induce damage (Taylor, 1990). Most of the diseases caused by nematodes are debilitating. However, when they interact with other pathogenic organisms the disease picture is drastically altered. It changes from debilitating to annihilating (Powell, 1963, 1971 a, b, 1979; Bergeson, 1972; Lamberti and Tayler, 1979; Haseeb, 1983; Sasser, 1989; Back *et al.*, 2002).

Most commonly grown vegetable crops in different regions of the world are severely attacked either by one or more species of plant parasitic nematodes and fungi. A combination of fungi and nematodes often results in a synergistic interaction, wherein the crop losses are greater than would be expected from invasion by either of the pathogen alone (Franc and Wheeler, 1993, Luc *et al.*, 2002; Back *et al.*, 2002).

As early as 1892, Atkinson recognized the ability of the nematodes to interact with fungi. He observed that infection by root-knot nematodes increased the severity of Fusarium wilt in cotton. Since then interest has been generated in the study of plant parasitic nematodes, particularly root-knot nematodes, in fungal disease complexes on a number of crops (Pitcher, 1965, 1978; Haseeb, 1983; Powell, 1963, 1971a, 1979; Bergeson, 1972; Garcia and Mitchell, 1975; Golden and Van Gundy, 1975; Orion and Krikum, 1976; Sidhu and Webster, 1977; Wallace, 1978; Webster, 1985; Griffin, 1986; Mai and Abawi, 1987; Walker *et al.*, 1999; Back *et al.*, 2002; Haseeb, 2003, 2004; Haseeb *et al.*, 2005 d).

The vast arsenal of fungicides and nematicides with varied spectra of activity has undoubtedly helped in minimizing the crop losses caused by various nematode-fungal disease complexes and in maintaining the sustainability in agriculture (Wright, 1981; Abu-El-Amayam *et al.*, 1985; Thind and Chahal, 2002). The use of fungicides and

nematicides in India on per hectare basis is far less compared to USA, Japan and European countries. However, the figures over the years show an increase in consumption of fungicides from 16,365 million tonnes in 1988 to 27,152 million tonnes in 1998 (Sharma and Sharma, 1999).

Mancozeb, copper oxychloride and carbendazim occupy most of the fungicide market in India. For the past more than 20 years, quite a good number of groups of systemic fungicides, such as benzimidazoles, thiophanates, carboxanilides, organophosphates, triazoles, morphalines, pridines, phenylalamides, alkylphosphates, tricyclazole, etc., are being used in India for controlling various crop diseases. However, their frequent and indiscriminate use often leads to atmospheric pollution and development of pesticide resistance in pathogens (Delp, 1980; Brent and Hollomon, 1998; Thind and Chahal, 2002). In this context, innovative approaches with limited use of chemicals which are ecology conscious and environment friendly are coming up as alternative strategies for disease management (Chet, 1987; Weller *et al.*, 2002).

Amendment of soil with decomposable organic matter such as oil cakes, dry leaves, seeds, seed kernel, seed coat and seed powder etc., for the control of plant pathogens, is an age old practice. It has been recognized as the most efficient method of changing soil and rhizospheric environments, thus adversely affecting the life cycle of pathogens and enabling the plant to resist attack of pathogens through better vigour and/or altered root physiology (Sayre *et al.*, 1964; Singh and Sitaramaiah, 1973; Rodriguez-Kabana, 1986; Alam, 1990; Haseeb, 1983, 2003, 2004). Among various organic additives, different plant parts and products of neem are of great importance in controlling the disease as these contain several active principles, which are responsible for fungicidal and nematocidal activity (Narayanan and Ayer, 1967; Barak and Chakraborty, 1969; Singh and Sitaramaiah, 1973; Rodriguez-Kabana, 1986; Alam, 1990; Haseeb, 1983, 2003, 2004).

In recent years, efforts have been made to restrict the use of chemicals to protect the environmental degradation. The use of non-chemical means of management of plant pathogens was advocated much while developing integrated pest management (IPM) for combating the diseases and pests. In this regard the use of biocontrol agents proved to be one of the most important component of IPM, which has a bright future for sustainable

agriculture. So far, more than 100 biocontrol agents belonging to fungi, bacteria, viruses, nematodes, protozoa, etc. have been described, characterized and tested to combat various diseases and to improve agricultural productivity (Baker and Cook, 1974; Kloepper *et al.*, 1980; Rodriguez-Kabana *et al.*, 1984; Jatala, 1986; Chet, 1987; Kerry, 1990; Stirling, 1991; Dickson *et al.*, 1994; Hoitink and Boehm, 1999; Harman *et al.*, 2004). Among these, *Trichoderma* spp. and *Pseudomonas* spp. have been used as biocontrol agents against several soil borne plant pathogens (Baker, 1968; Baker and Cook, 1974; Papavizas and Lumsden, 1980; Windham *et al.*, 1986; Chet, 1987; Weller, 1988; Kerry, 1990; Stirling, 1991; Cook, 1993; Harman *et al.*, 2004). For many years, *Trichoderma* species have been known to produce a wide range of antibiotic substances, to parasitize pathogenic fungi and to compete with microorganisms for nutrients and/or space (Elad, 1995; Harman *et al.*, 2004).

Similarly, in recent past, the plant growth promoting rhizobacterium, *P. fluorescens* has received considerable attention for their inherent quality to produce antibiotics, hydrogen cyanide and siderophores, which are involved in suppression of plant root pathogens (Kloepper *et al.*, 1980; O'Sullivan and O'Gara, 1992). The simple nutritional requirement and the ability to use many carbon sources that exude from roots and to compete with indigenous microflora, may explain their ability to colonize the rhizosphere (Weller, 1988; Mazzola and Cook, 1991).

Scanning of literature reveals that considerable work has been carried out by several workers on the effect of root-knot nematodes on the growth and yield of various vegetable crops (Dasgupta, 1997; Upadhyay and Dwivedi, 2000; Luc *et al.*, 2002; Haseeb, 2003; Haseeb *et al.*, 2005 b; Kumar and Haseeb, 2006 a, b); the effect of *Fusarium* spp. (Beckman, 1987; Elias and Schneider, 1991; Alabouvette *et al.*, 1998; Weller *et al.*, 2002; Walker, 2004; Agrios, 2005; Haseeb *et al.*, 2005 c; Sant *et al.*, 2008). Similarly, the effect of *Rhizoctonia* spp. (Stephens *et al.*, 1982; Walker, 2004; Tyagi *et al.*, 2005; Sarkar and Saxena, 2007) alone and as disease complexes of *Meloidogyne* and *Fusarium* (Bergeson *et al.*, 1970; Sidhu and Webster, 1974, 1977, 1983; Carter *et al.*, 1977; Fatah and Webster, 1983, 1989; Parveen and Ghaffar, 1998; Jain *et al.*, 2001; Haseeb, 2003; Haseeb *et al.*, 2005 d; Samuthiravalli and Sivakumar, 2008) is well documented. The *Meloidogyne*-*Rhizoctonia* interaction effect has also been studied

(Khan and Muller, 1982; Haseeb, 1983; Evans and Haydock, 1993; Haseeb *et al.*, 2004). Efforts have also been made to manage the root-knot nematodes alone (Haseeb, 1992, 1994; Haseeb and Butool, 1993; Butool *et al.*, 1998; Rajendran and Saritha, 2005; Haseeb and Shukla, 2007; Singh *et al.*, 2008), *Fusarium* spp. alone (Sivan and Chet, 1986; Kapoor and Kumar, 1991; Padmodaya and Reddy, 1998; Cheuk *et al.*, 2003; Bharath *et al.*, 2005; Haseeb *et al.*, 2005 a, 2006 a; Haseeb and Kumar, 2006, 2007 a) and *Rhizoctonia* spp. alone (Bari and Mukhopadhyay, 1988; Ho *et al.*, 1992; Varshney and Chaube, 1999; Pande and Chaube, 2003; Saxena *et al.*, 2004; Sarkar and Saxena, 2007; Devi *et al.*, 2008) affecting various vegetable crops. However, very meager work has been carried out on the management of *Fusarium-Meloidogyne* (Stephan *et al.*, 1996; Haseeb *et al.*, 2004, 2005 c, d, 2006 b, 2007 a; Haseeb and Kumar, 2005, 2008 a, c), and *Rhizoctonia-Meloidogyne* disease complex (Walia *et al.*, 1994; Arya and Saxena, 1998; Chaitali *et al.*, 2003; Haseeb and Kumar, 2008 b) on vegetable crops including tomato.

The exact identification of plant parasitic nematodes and soil borne fungi responsible for decline in yield of tomato grown in western Uttar Pradesh is still lacking and no systematic work has so far been done regarding the effect of *Meloidogyne-Fusarium-Rhizoctonia* disease complex on the yield of tomato in western Uttar Pradesh. Similarly, efforts have also not been made to manage the disease complex through integrated approach. Keeping in view the extent of losses caused by this disease complex on tomato, absence of effective management technology and the ever increasing demand for tomato, it is urgently required to assess the losses and to develop the package of practices for the management of this disease complex through integrated approach. Therefore, following experiments were planned and conducted to develop integrated disease management package for increased the production of tomato.

1. Survey for the occurrence, distribution and identification of nematode and fungi, causing disease complex in tomato, and for collection of naturally occurring fungal and bacterial antagonists from western districts of Uttar Pradesh
2. Pathogenicity test of *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* alone on tomato cv. K-25 under pot conditions
3. Interactive effect of *M. incognita*, *F. oxysporum* and *R. solani*, alone and in combination on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

4. In-vitro, evaluation of biocontrol agents, organic amendments and pesticides for their efficacy against *F. oxysporum* and *R. solani* alone
5. Comparative efficacy of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* alone and in combinations, on tomato cv. K-25 under pot conditions
6. Compatibility test, *in-vitro*, among fungal and bacterial biocontrol agents and thereof with pesticides and organic amendments
7. Efficacy of biocontrol agents, organic amendments and pesticides alone and in combination, against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in nursery under sick plot conditions
8. Evaluation of various treatments of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in sick field conditions

CHAPTER – 2

REVIEW OF LITERATURE

The term SURVEY is derived from two Latin words ‘Sur’ and ‘Video’ means to over and see respectively i.e. a general survey or inspection or collection of data for mapping. Surveillance denotes repeated or sequential survey of the same place or locality for taking observations to see the changes or fluctuations in the object of study. The type of survey may be qualitative - involving the identification of different species present in that area or quantitative - involving the estimation of population of one or more species present in that area. The survey may be extensive - when it covers a vast area for study or intensive - when more accurate detailed knowledge is required. Survey may be locality-wise, host-wise or pathogen-wise according to the requirement.

Plant parasitic nematodes are known to affect the production and economy of the crops in a number of ways (i) by reducing quality and quantity of crops (ii) increase need of additional fertilizer, water and application of nematicides and (iii) impeding production and trade by phytosanitary regulations (Weischer, 1967).

A worldwide survey covering 75 countries involving 371 nematologists revealed that *Meloidogyne*, *Pratylenchus*, *Heterodera*, *Ditylenchus*, *Globodera*, *Tylenchulus*, *Xiphinema*, *Radopholus*, *Rotylenchulus* and *Helicotylenchus* are the most damaging genera of plant parasitic nematodes occurring in these countries (Sasser and Freckman, 1987). Among these, *Meloidogyne* species have been considered most important because of their highly specialized parasitic mode of life, world wide distribution, extensive host range and ability to cause infection on almost all types of plants like vegetables, legumes, cereals, horticultural, plantation, medicinal and aromatic plants (Webster, 1972; Sitaramaiah, 1984; Haseeb, 1992, 1994, 2003; Luc *et al.*, 2002; Haseeb and Shukla, 2007).

As early as 1855, Berkley for the first time reported root-knot nematode disease of cucumber grown in glass house in England, he named the nematode as ‘vibrios’ and the disease as ‘excrecence’. In India, this disease was first reported by Barber (1901) on tea in Kerala.

An extensive survey of *Meloidogyne* spp. in the north-western frontier province of Pakistan was undertaken during 1983 through 1986. A total of 494 samples comprising 58 species of plants were collected from 146 localities in all 14 districts. It was noticed that only tomato, okra and brinjal were infested with all the four species of *Meloidogyne* viz., *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* (Gul and Saeed, 1990).

Mathur and Khera (1991) surveyed the Chandigarh city to determine the infestation of plant parasitic nematodes associated with cereal and vegetable crops. They found that the vegetable crops were mostly infested with *M. incognita* besides other plant parasitic nematodes.

Rana and Ali (1992) surveyed the 26 host plants comprising 19 vegetables and 7 legumes from Chitwan, Nepal to determine the infestation of *Meloidogyne* spp. They reported that *M. incognita* was the most common and predominant species found on 56% of the crops, followed by *M. arenaria* and *M. javanica* with 37% and 23% crop infestation, respectively.

Sharma *et al.* (1993) surveyed different ginger growing localities from Sirmour, Solan, Shimla and Kangra districts of Himachal Pradesh to determine the prevalence of species of root-knot nematode and lesion nematode. They found that *M. incognita* and *P. coffeae* were associated with rhizomes infected with fungus and their rhizospheric soil. However, these nematodes were absent in the samples collected from Shoghi, Chausa and Rajpura.

Sorribas and Verdejo-Lucas (1994) conducted the survey to determine the frequency and abundance of *Meloidogyne* spp. in 45 tomato production sites at Barcelona, Spain and reported that *Meloidogyne* spp. occurred in 49% of the sites sampled. Preplant population densities ranged from 10-220 juveniles (J_2)/250 g soil and final population densities from 20-1530 J_2 /250 g soil. Final population densities were higher in open fields than in green houses but initial population densities were higher in green houses than in fields.

Yamamoto and Toida (1995) surveyed 19 fields growing various vegetables and ornamental plants at Torniura, Japan for the association of plant parasitic nematodes. They found that *Meloidogyne* spp. was mainly associated with these crops.

Overstreet and McGawley (1996) conducted a survey during 1994 and 1995 throughout the cotton producing areas in Louisiana, USA and reported that *R. reniformis* was widespread throughout the state and found in 56% of the 200 samples collected. *Meloidogyne* spp. was found in 27% of the samples and the population of *Helicotylenchus* spp. was also encountered in 68% of the samples.

Hankins *et al.* (1997) surveyed 60 cotton fields in the non-delta region of Mississippi, USA for the association of plant parasitic nematodes and reported that *Meloidogyne* spp. was present only in 10% of the sites sampled.

D' Souza *et al.* (1998) collected 189 soil and root samples from vegetable crops and medicinal plants from 28 different counties of Minas Gerais, Sao Paulo, Rio Grande do Norte, Para and Pernambuco states, Brazil. Seventeen nematode genera were found associated with 103 plant species. *Helicotylenchus* (31.2%) was the most predominant nematode found in the samples followed by *M. incognita* (27.5%), *M. javanica* (24.2%), *P. brachyurus* (16.4%), *Crictonemella* (11.1%), *C. sphaerocephala* (10.0%), *Aphelenchus* (6.3%), *P. zeae* (5.8%), *M. hapla* and *C. xenoplex* (5.2%) and *Tylenchus* and *Rotylenchulus* (1.0%), respectively.

Ornat and Verdejo-Lucas (1999) surveyed 50 plastic green houses and 30 fields to determine the association of different species of *Meloidogyne* with vegetable crops grown in El Maresme County, Barcelona, Spain. They reported that *Meloidogyne* spp. (*M. arenaria*, *M. incognita*, *M. javanica*) was present in 34% samples and were more frequent and abundant in green houses than in fields.

Dautova and Gommers (2000) surveyed several areas of the Republic of Macedonia to determine the occurrence of *Meloidogyne* spp. and found *M. incognita* (47.9%) as most prevalent species followed by *M. javanica* (35.6%), *M. arenaria* (13.7%) and *M. hapla* (2.7%), respectively. Mixture of species was present in nearly all locations.

Devrajan (2001) surveyed banana and the rice fields of 55 villages in Tamirabarani river basin covering Tirunelveli and Thoothikudi districts of Tamil Nadu. He reported that *Hel. multicinctus* was found more frequently than *P. coffeae*, *R. similis*, *M. incognita* and *Hoplolaimus* spp. in banana. While in rice fields, *Hirschmanniella*

oryzae was found more frequently followed by *Hoplolaimus* spp. and *Tylenchorhynchus* spp., respectively.

A survey was conducted by Chakraborti (2002) during four seasons to record the incidence of *M. incognita* on onion seedlings in West Bengal. He reported that on the average, 10-20% of the plants were infected after 20 days of transplanting.

Haseeb (2003) conducted surveys for the association of the *Meloidogyne* spp. with brinjal, chilli and tomato crops in Aligarh, Agra, Bulandshahar, Hathras and Mathura districts of Uttar Pradesh and reported *M. incognita* as the most severe constraint in the cultivation of these crops. Severe stunting and wilting of plants was observed in farmer's field and the diseased plant had very severe galling on their roots.

Ravichandra and Krishnappa (2004) conducted intensive survey during September through December 2000 to determine the prevalence and distribution of phytopathogenic nematodes associated with major vegetable crops at 3 taluks of Mandya district, Karnataka and reported that *M. incognita*, *M. javanica*, *Tylenchorhynchus* (*Trh.*) *vulgaris* and *Helicotylenchus* (*Hel.*) *indicus* were most commonly encountered species of plant parasitic nematodes and also have the potential of causing damage in the surveyed area as evident from their high frequencies, densities and prominence values.

Senthamizh *et al.* (2005) conducted a survey of plant parasitic nematodes associated with groundnut in Erode district of Tamil Nadu. They reported that *Criconemoides curvatum*, *Heli. dihystra* and *Hop. seinhorsti* present in soil. While *P. brachyurus*, *M. arenaria*, *M. incognita* and *Rotylenchulus* (*Roty.*) *reniformis* present in soil and root samples. *P. brachyurus* and *Roty. reniformis* were frequently encountered and were present in more than 50% samples.

Yadav *et al.* (2006 a) surveyed the brinjal growing areas of Allahabad district of Uttar Pradesh, India to determine the prevalence and distribution of plant parasitic nematodes. They reported that 6 genera of plant parasitic nematodes were found in the soil samples collected from the rhizosphere of brinjal. *Meloidogyne* spp. was the most prevalent nematode followed by *Hop. indicus* and *Trh. brassicae*. However, *M. incognita* was found in all samples with high population.

Roy *et al.* (2007) surveyed the plant parasitic nematodes associated with leguminous vegetable crops viz., cowpea, dolichos bean, french bean and pea in seven

districts of West Bengal. They found the occurrence of 17 species of plant parasitic nematodes belonging to 8 genera. Highest frequency of occurrence was recorded in *Roty. reniformis* followed by *Meloidogyne* spp., *Tylenchorhynchus* spp., *Criconea* spp., *Pratylenchus* spp., *Hoplolaimus* spp., *Helicotylenchus* spp. and *Hirschmanniella* spp. However, *Meloidogyne* spp. was found in all the root samples in all the seven districts on cowpea, dolichos bean, french bean and pea.

Sudheer *et al.* (2008) surveyed the different economically important crops growing fields in 9 districts of Andhra Pradesh, India to determine the distribution of plant parasitic nematodes. They reported that out of 1478 samples collected, 1152 were found to contain plant parasitic nematodes belonging to 15 genera and 48 species. *M. incognita* was predominant and recorded in majority of the samples associated with pomegranate, tobacco, betelvine, turmeric, chrysanthemum, banana, tomato, citrus, grapevine, chilli, brinjal, gourds, mulberry and okra.

Fungi are one of the most important pathogens causing severe yield losses to agricultural crops (Agrios, 2005). The great potential to adopt a wide range of conditions makes them an important group of pathogens (Swarup, 1990). More than 10,000 species of fungi are found to be capable of causing plant diseases. Almost each and every crop is parasitized by one or more species of fungi and each of the parasitic fungi often attack various crops (Agrios, 2005). Ten fungal genera have been recognized, playing a major role in the root disease complexes causing seed decay, damping-off, root-rot, seedling blight, collar, crown, foot-rot and wilt (Cook and Baker, 1983). Among them, species of *Fusarium* and *Rhizoctonia* are the non-specific and most destructive plant pathogens occurring globally and causing various maladies starting from seed decay, damping-off, root and stem rots, wilt, canker, sheath blight and ear rot in various plants (Parmeter, 1970; Sen, 2000; Haseeb, 1983; Haseeb, 2003, 2004).

Brammall and Lynch (1990) surveyed green houses growing tomatoes for the first time in New Brunswick, Canada to determine the pathogen associated with crown and root rot of tomato. They identified the pathogen as *F. oxysporum* f. sp. *radicis-lycopersici*. They have also established the pathogenicity of identified pathogen on tomato. Further, they have reported that tomato cultivars WR25, Walter and MR13 as susceptible and Cr6 as resistant upon the screening studies.

Hartman and Fletcher (1991) reported that *Fusarium* crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici* was found in UK during 1988 and 1989 mainly in rockwool-grown tomato crops and about 14% tomatoes were affected by this pathogen.

Reddy *et al.* (1991) surveyed the chickpea growing areas of Myanmar, Sagaing, Bago, Central and Lower Myanmar to study the fungal disease associated with the crop. They found that *Fusarium* wilt was the major disease in all the surveyed areas followed by root rot caused by *Macrophomina phaseolina*.

Etebarian (1992) reported that *Fusarium* wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* is one of the most prevalent and destructive disease of tomato in the Varamin area of Iran. The mean percentage of *Fusarium* wilt infection in tomato fields was recorded approximately 27% during 1983 through 1989. However, in some parts of the Varamin area, entire fields of tomato were killed before the crop was harvested.

Cartia and Asero (1994) surveyed 42 farms growing 6 different tomato varieties in plastic houses of Sicily, Italy. They reported that out of 149 plastic houses, 36 were affected with *Fusarium* wilt and all varieties were equally susceptible to *F. oxysporum* f. sp. *radicis-lycopersici*. Damage was greater in winter grown tomatoes with an incidence of affected plants varying from 15 to 100%.

Franceschini *et al.* (1996) carried out a 4-year survey during 1992 through 1995 in 1299 green houses in southern Sardinia, Italy to study the etiology of the crown and root rot disease of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici*. The disease occurred in 67% of cultivations made in soil and in 43.5% of soilless cultivations, with the percentage of infected plants ranging from 8.5 to 15.8%.

Dolar (1996) surveyed the main chickpea growing areas in Ankara province, Turkey during 1992 through 1994 to determine the fungal diseases. He reported that *F. oxysporum* f. sp. *ciceri* (49%) was the most common wilt and root-rot pathogen which cause economic losses to the crop yield followed by *F. solani* (34%). He also reported that *F. moniliforme*, *F. equiseti*, *F. sambucinum* and *M. phaseolina* were also associated with chickpea wilt and root-rot.

Javed *et al.* (1997) recorded thirteen fungi of 8 genera in varying frequencies from the seed samples of onion collected from various locations in Punjab, Pakistan. They isolated five species of *Fusarium* viz., *F. moniliforme*, *F. equiseti*, *F. oxysporum*, *F.*

pallidoroseum and *F. solani* from the seed samples of onion. They also found *Aspergillus* sp., *Rhizopus* sp., *Alternaria alternata*, *Drechslera siccans*, *D. tetramera*, *Mucor* sp., *Arthrobotrys* sp. and *Curvularia lunata*.

Singh and Tripathi (1999) surveyed twenty five villages belonging to Azamgarh, Basti, Deoria, Gorakhpur and Maharajganj districts of Uttar Pradesh for occurrence of wilt of lentil caused by *F. oxysporum* f. sp. *lentis*. They reported that the maximum wilt occurrence was observed in Gorakhpur (23.0%) followed by Maharajganj (19.6%), Azamgarh (17.6%), Deoria (16.7%) and Basti (12.5%), respectively.

Andrade and Michereff (2000) surveyed the disease intensity of *F. oxysporum* f. sp. *lycopersici* on tomatoes in 50 planting areas of Camocim de Sao Felix, Agreste, Pernambuco, Brazil. Studies indicated that the highest disease prevalence was 72% with an average incidence of 17.15%.

Bost (2001) reported that tomato plants in several commercial fields in southeastern and eastern Tennessee, USA exhibited symptoms of Fusarium wilt. All cultivars on which symptoms were observed were classified as resistant to races 1 and 2 of the causal fungus, *F. oxysporum* f. sp. *radicis-lycopersici*. Race 3 has been reported from several areas but not from Tennessee.

Arora and Gupta (2002) surveyed root rot and wilt disease of pea caused by *F. oxysporum* f. sp. *pisi* in different districts of Haryana. They reported that the root rot and wilt disease of pea was prevalent in all the localities and the maximum disease incidence was recorded in Hisar (47.7%) and minimum in Faridabad (2.5%). The disease incidence was recorded more in the crop sown by the end of September or first week of October.

Stirling and Ashley (2003) surveyed the soilborne diseases in 34 tomato fields in the Bundaberg region of Queensland and reported that the incidence of *F. oxysporum* f. sp. *lycopersici* race 3 was relatively low. Less than 1.3% of plants were affected in fields fumigated with methyl bromide or metham sodium, or in crops grown in rotation with sugarcane. In non-fumigated fields that had recently grown tomato, high disease levels (4.4 and 8.6% affected plants) were observed in only two out of eight fields.

Mathur *et al.* (2004) surveyed the farmer's field of chilli near Jaipur city to study the rhizosphere microflora in chilli crop. They found that species of *Rhizoctonia* (31.80%), *Aspergillus* (29.30%), *Alternaria* (13.94%), *Rhizopus* (4.07%),

Helminthosporium (4.07%), *Trichoderma* (4.49%), *Fusarium* (1.75%), *Acremonium* (5.26%) and sterile mycelium (5.46%) were common in the rhizosphere soil of chilli crop. Among *Rhizoctonia* spp., *R. solani* (24.03%) occupied the first place followed by *R. bataticola* (*M. phaseolina*) (7.77%). *R. solani* was more common in summer season and caused severe losses to the summer crop grown during zaid.

Verma and Dohroo (2005) surveyed the different locations of Himachal Pradesh to collect the seeds of pea infected with fungal pathogens. They reported that twenty two fungi were associated with pea seeds. *F. oxysporum*, *A. alternata* and *P. ultimum* were found to be internally seed borne. While *Aspergillus* (*As.*) *flavus*, *As. niger*, *Peni. chrysogenum*, *Rhizopus nigricans*, *G. virens*, *Rhizoctonia* sp. and *Penicillium* sp. were found to be externally seed borne.

John *et al.* (2006) surveyed the 200 orchards which includes 80 in Allahabad and 120 in Kaushambi districts. They reported that *F. oxysporum* f. sp. *psidii* and *V. albo-atrum* were predominantly involved from the wilted guava roots. *Fusarium* sp. dominated in Allahabad, while *V. albo-atrum* dominated in Kaushambi district. The biocontrol agents viz., *T. viride* and *As. niger* were also found in these districts.

Srivastava *et al.* (2008) surveyed the each blocks of Baharaich district to determine the wilt incidence of pigeonpea. They reported that *Fusarium* wilt was present in all 14 blocks with the range from 5 to 18% incidence. Highest range (13-18%) and average incidence (10%) was recorded in Mahri block.

In the soil ecosystem numerous interrelationships between microbial communities, host plant and pathogens take place (Vilich and Sikora, 1998). It is not surprising that many investigators have concluded that the majority of the root diseases have a complex etiology (Wallace, 1978; Grogan, 1981). As early as 1931, Fawcett stated that "nature does not work with pure cultures" and that most of the plant diseases, particularly root diseases are influenced by associated microorganisms including plant parasitic nematodes. Root infection by one pathogen may modify the host response to subsequent infections by pathogens or saprophytes (Mai and Abawi, 1987). Nematode particularly *Meloidogyne* spp. interact with these microorganisms, especially fungal pathogens such as *Fusarium* spp. and *Rhizoctonia* spp., resulting in an increase in losses to plants. Various workers have written several reviews on this subject (Miller, 1965;

Pitcher, 1965; Powell, 1971 a, b; Khan and Muller, 1982; Haseeb, 1983; Webster, 1985; Swarup, 1990; Khan, 1993; Back *et al.*, 2002; Luc *et al.*, 2002).

Ali and Venugopal (1993) surveyed the cardamom nurseries of various ages in Karnataka for identifying the nematode and fungus species causing the damage to the seedlings. They revealed that *M. incognita* and *R. solani* / *Pythium* spp. were found to be associated with all ages of the seedlings. In old nursery beds severe incidence was recorded within the primary nursery beds. Other fungi isolated from diseased seedlings were *S. rolfsii*, *Cylindrocarpon* sp. and *Fusarium* sp.

Devi (1994) conducted a survey in Allahabad in pigeonpea growing fields to determine the cause of yield reduction. Studies indicated that high population of *Heterodera* (*Het.*) *cajani*, *R. reniformis*, and *M. incognita* and *F. udum* was isolated from such fields.

Park *et al.* (1995) surveyed for three years in vinyl houses in Kyeong-Buk province, Korea growing vegetables to study the causal agents of disease complex particularly *M. incognita* and other soil borne pathogens. They reported that the severity of disease increase with the co-existence of *M. incognita* and *Fusarium* spp. Studies also indicated that almost all the test vegetables were invariably infested with root-knot nematode and wilt fungi.

Suliman *et al.* (1997) surveyed 2 fields of muskmelon in Sudan to determine the cause of the wilt disease complex. Observations in the Galia fields revealed that out of the total plant surveyed, 24.4% plants had symptoms of wilt, of which 71.3% had symptoms typical of those caused by *Fusarium*. Similarly in the Ananas field, 21.18% had wilt symptoms, of which 61% were typical of those caused by *Fusarium*. Besides, *Fusarium*, the *M. incognita* was also invariably associated with wilted plants.

Srivastava *et al.* (1998) conducted the disease survey in the main ginger growing areas in Sikkim. Thirty nine microorganisms were found associated with ginger either in the field or in storage, of which *M. incognita*, *P. coffeae*, *Ralstonia solanacaerum*, *F. oxysporum*, *P. aphanidermatum* were most potent pathogens causing yellows/wilt, soft rot and dry rot, respectively in the field or in storage.

Haseeb *et al.* (2002) surveyed the farmer's fields growing chilli at different localities of Agra, Aligarh, Bulandshahar, Hathras and Mathura districts of Uttar Pradesh

for the association of plant parasitic nematodes and wilt fungi. Highest severity of *M. incognita* infection was observed at different localities in Bulandshahar followed by Mathura, Hathras, Aligarh and Agra, respectively. Infection of roots by *F. oxysporum* was highest in Hathras followed by Aligarh, Mathura, Bulandshahar and Agra, respectively. Extent of yield loss was highest (24%) at Mathura followed by Hathras, Bulandshahar, Aligarh and Agra, respectively.

Shukla and Haseeb (2002) surveyed the pigeonpea growing fields of Agra, Aligarh, Bulandshahar, Hathras and Mathura districts of Uttar Pradesh to determine the incidence of nematode-fungus wilt disease complex of pigeonpea. They reported that *Het. cajani* was the most prevalent nematode species along with the infestation of *Fusarium* spp. associated invariably with the infested roots. However, the population of *Trh. brassicae*, *Hop. indicus* and *M. incognita* were very high at some locations. Severity of stunting, yellowing and wilting of plants was directly proportional to the population of pigeonpea cyst nematode and wilt fungus. Highest population of *Het. cajani* was found in Bulandshahar followed by Agra, Aligarh, Mathura and Hathras, respectively.

Parameshwari and Lingaraju (2004) surveyed the 33 gardens in 14 villages of three major betelvine growing districts of northern Karnataka to determine the nematode-fungal wilt disease complex. They reported that wilting due to foot rot/collar rot causing fungi viz., *M. phaseolina*, *S. rolfsii* and *F. solani* along with the *Meloidogyne* spp. was noticed in many locations. Rhizoctonia (collar) rot was widely prevalent in the areas surveyed (18.1%). However, *S. rolfsii* and *F. solani* were also associated with foot rot. Root-knot disease showed an incidence of 18.2%. Highest disease infestation was found in Haveri district, followed by Koppal and Bangalkot districts. *Pratylenchus* spp., *Helicotylenchus* spp., *Radopholus* spp. and various *Dorylaimid* genera were also found at different localities.

Mir *et al.* (2005) surveyed the fungal and nematode diseases associated with Rabi season vegetables (potato, cabbage, chilli and brinjal) and Kharif season cereals (rice and wheat) in Chaka block of Allahabad district, Uttar Pradesh, India. They reported that early blight of potato was found to be predominant with 37% disease intensity followed by leaf spot of rice 31.6%, Fusarium wilt of tomato 29%, brown rust of wheat 27%, false smut of rice 26%, leaf spot of brinjal 24%, root-knot of rice 22%, loose smut of wheat

22%, root-knot of tomato 21%, root-knot of brinjal 19%, ear-cockle of wheat 19%, verticillium wilt of brinjal 15%, respectively.

Root-knot nematodes belonging to the genus *Meloidogyne* are the most numerous among all the plant parasitic nematodes and stands first in causing crop damage and yield losses. They infect all types of plants like legumes, cereals, vegetables, horticultural crops, medicinal and aromatic plants etc. (Webster, 1972; Sasser and Carter, 1985; Haseeb, 1992, 1994; Luc *et al.*, 2002; Haseeb and Shukla, 2007).

The ability to predict losses expected from a given nematode population is essential in making management decisions (Barker *et al.*, 1985). It is established that increasing nematode population densities can progressively decrease crop performance and there is a minimal threshold density below, which no measurable loss in yield occurs. Studies pertaining to such relationship have been conducted with several nematode-crop/plant systems. Most of the informations are based on the studies carried out in pots or micro-plot experiments (Shaffie and Jankins, 1963; Appel and Lewis, 1984; Di Vito *et al.*, 1985; Haseeb, 1992, 1994, 2003, 2004; Singh and Nath, 1996; Mahapatra *et al.*, 1999; Singh and Hasan, 2002; Haseeb and Shukla, 2007).

Pankaj and Siyanand (1990) studied the effect of initial inoculum levels (Pi) viz., 0, 10, 100, 1000, and 10,000 J₂ of *M. incognita*/kg soil on bittergourd and round melon under pot conditions and reported that significant reduction in plant growth was observed at all the Pi. The damaging threshold in bittergourd was found to be 1 J₂/g soil as against 10 J₂/g soil in round melon.

Bhagwati (1991) established the pathogenicity of *M. incognita* on pea variety Bonneville under glass house conditions. He reported that maximum number of galls and egg masses were found at 1000 J₂/500 g soil. The reproduction rate of nematode was observed inversely proportional to the initial inoculum densities.

Chan and Lopez (1992) studied the effect of different initial population densities of *M. incognita* on the growth of tomato cv. Tropic under green house conditions. They reported that 200 eggs/100 g soil was the tolerance limit. Highest reproduction rate was observed at the Pi of 600 eggs/100 g soil. Root-knot index varied among the Pi and there was no correlation between reproduction rate and root-knot index.

Bawage *et al.* (1993) determined the effect of *M. incognita* on seedling emergence, development and vigour of tomato by inoculating trays of tomato seeds with 0, 5, 10, 15 or 20 J₂/tray. All three parameters were significantly suppressed by *M. incognita* except at 0 and 5 J₂/tray and reduction increased with an increase in nematode populations.

Hazarika and Phukhan (1994) determined the effect of different Pi (0, 10, 100, 1000, 5000 or 10,000 J₂/pot) of *M. incognita* on brinjal var. JC-1. They found significant reduction in plant height, root and shoot weights of plants at an inoculum level of 1000 nematodes/plant.

Gupta *et al.* (1995) studied the effect of various Pi viz., 0, 10, 100, 1000, 2000, 5000 and 10,000 J₂ of *Meloidogyne* spp./kg soil on some cucurbitaceous crops. They found significant reduction in the growth of all the crops at Pi of 1000 J₂/pot. Gallings was found maximum at highest Pi.

Singh and Nath (1996) studied the effect of various Pi viz., 0, 10, 100, 1000, 10,000 J₂ of *M. incognita*/500 g soil on the growth of papaya under pot conditions. They reported that with the increase in inoculum level of the nematode, there was a corresponding decrease in plant growth characters except at Pi of 10 J₂/500 g soil. An initial population of 1000 J₂/500 g was found as damaging threshold level for papaya plant.

Makhnotra and Khan (1997) conducted an experiment to assess the yield losses in ginger due to *M. incognita* under field conditions. Studies revealed that 20 percent loss in ginger yield was obtained at Pi of 200 J₂/200 g soil.

Borah *et al.* (1998) studied the pathogenicity of *M. incognita* on papaya with 5 Pi viz., 100, 1000, 3000, 5000 and 10,000 J₂/3 kg soil under pot conditions. They reported that there was a progressive decrease in growth of the plant with the increase in inoculum level. An initial inoculum level of 3000 J₂/3 kg soil was found to be the damaging threshold level of *M. incognita* on papaya.

Mahapatra *et al.* (1999) reported that a significant reduction in shoot and root weight of pointed gourd was observed at an initial inoculum density of 1000 J₂ of *M. incognita*/kg soil under pot conditions. The rate of nematode reproduction was density

dependent, the maximum being at the lowest inoculum density, and the minimum at the highest density.

Sharma and Siddiqui (2000) studied the effect of different inoculum levels viz., 0, 10, 100, 1000 and 10,000 J₂ of *M. incognita*/plant on the growth and development of pea cv. Bonneville under green house conditions. They reported that significant reduction in plant growth characters were observed at or above 1000 J₂/plant. The number of galls and egg masses/plant increased progressively with the increase in Pi from 10 to 10,000 J₂/plant. However, eggs and J₂/egg mass and reproduction factor declined with an increase in Pi.

Singh and Hasan (2002) studied the pathogenic potential of *M. incognita* on bottle gourd with different inoculum levels viz., 10, 100, 1000 and 10,000 J₂/kg soil under pot conditions. They reported that the plant growth was adversely affected at Pi of 100 J₂/kg soil and beyond. The development of root galls, egg masses and nematode population increased with the increase in Pi.

Khan (2003) determined the effect of different inoculum levels viz., 10, 100, 1000, 5000 and 10,000 J₂ of *M. incognita* race-2/500 g soil on onion under pot conditions. They reported that *M. incognita* race-2 was pathogenic to onion even at an inoculum level of 10 J₂/500 g soil and cause significant reduction in weight of onion bulb.

Kumar and Pathak (2004) studied the effect of *M. incognita* on spinach beet and fenugreek. They reported that *M. incognita* adversely affected the seed germination and seedling emergence of spinach beet at 500 J₂/kg soil in both sterilized as well as in unsterilized soil. However, in fenugreek the significant reduction in germination started at 500 J₂/kg soil in both the soils but seedlings emergence was inhibited at 1000 J₂/kg soil in unsterilized soil while in sterilized soil it was at 500 J₂/kg soil. The significant reduction in plant growth characters of spinach beet and fenugreek was observed at 500 J₂/kg soil and above. There was an increase in number of galls and total nematode population with increase in inoculum levels from 50 to 5000 J₂/kg soil.

Haseeb *et al.* (2005 b) studied the effect of *M. incognita* at 0, 100, 250, 500, 1000, 2500, 5000 and 10,000 J₂/kg soil on root-knot development and plant growth on mungbean cv. ML-1108. They reported that significant reduction in all growth and yield

parameters was observed at and above Pi of 500 J₂/kg soil. Reduction in all growth and yield parameters increased with the corresponding increase in Pi.

Kumar and Haseeb (2006 a) studied the pathogenic potential of *M. incognita* on chilli cv. Jwala at different Pi viz., 0, 500, 1250, 2500, 5000, 7500 and 10,000 J₂/4 kg soil under pot conditions. They reported that reduction in all growth parameters increased with the corresponding increase in Pi. The significant reduction in all growth parameters was observed at the lowest Pi (500 J₂/4 kg soil) as compared to uninoculated control. Maximum reduction in number of fruits, fruit weight, shoot height, root length, shoot-root fresh and dry weights was 38.3, 40.0, 26.8, 33.5, 30.4, 33.8, 33.2 and 37.3% respectively at highest Pi (10,000 J₂/4 kg soil) as compared to uninoculated control. The final population of nematode in roots and soil and root-knot index were increased with the corresponding increase in Pi, while the reproduction factor was decreased. Maximum root-knot index (3.8) was observed at the highest Pi, whereas maximum reproduction factor (10.9) was observed at minimum Pi.

The fungus *Fusarium* occurs widely in nature as saprophytes in soil and decaying vegetables, some species are plant parasites, where specialized pathotypes may cause vascular wilt, stem rot, fruit rot and damping off diseases. Of the various species of *Fusarium*, *F. oxysporum* is one of the most ubiquitous soil fungus and a destructive plant pathogen of hundreds of hosts belonging to all the botanical families with the exception of Gramineae, causing chiefly wilt and root rots (Booth, 1971; Domsch *et al.*, 1980; Alabouvette *et al.*, 1998). *Fusarium* wilt is one of the most prevalent and damaging diseases of tomato wherever tomatoes are grown intensively (Beckman, 1987; Agrios, 2005). The disease was first reported in India from Pusa (Bihar) caused by *F. oxysporum* f. sp. *lycopersici* as the pathogen (Butler, 1918). Subsequently *F. solani*, *F. equiseti*, *F. semitectum* var. *majus*, *F. lateritium* (Verma, 1954); *F. semitectum* (Nedumaran and Vidyasekaran, 1982) have also been associated with tomato wilt.

El-razik *et al.* (1990) investigated the role of onion seeds in transmission of pathogenic fungi in Egypt. Of the 15 species from 10 genera isolated, only *F. oxysporum*, *F. moniliforme* and *R. solani* were pathogenic and caused pre and post emergence damping off.

Hartman and Fletcher (1991) determined the pathogenic potential of *F. oxysporum* f. sp. *radicis-lycopersici* on tomato. They reported that leaf and stem symptoms did not appear until the time of first fruit harvest even when the plants were inoculated at planting, first flowers or fruit set. Conidial inoculum at 10^6 cfu/plant applied at seed sowing killed 70-83% of tomato seedlings, whereas similar levels of inoculum applied to young plants caused root and basal stem decay, and eventually death but only after fruit harvest began. Disease incidence and symptom severity increased with inoculum concentration. Experimentally, the disease was more severe in peat or compost grown plants than in rock wool. Disease spread was only a few centimeters in 50 days in experimental rock wool-grown plants. All tomato cultivars tested were highly susceptible.

Yousef *et al.* (1992) established the pathogenicity of *Fusarium* spp. on different cultivars of tomato. Cultivar Castle Rock was found resistant whereas, cultivar Ace was highly susceptible. Disease severity was proportionately correlated with the concentration of fungal spores in the soil. Maximum infection of tomato wilt fungus was observed at 45-million cfu/kg soil under green house conditions.

Jimnez-Diaz *et al.* (1993) tested the pathogenic potential of 107 isolates of *F. oxysporum* f. sp. *ciceri* obtained from various countries on chickpea. They reported that disease was induced only by 45 isolates of the fungus.

Kusunoki and Tezuka (1994) determined the pathogenic potential of 4 isolates of *F. oxysporum* f. sp. *radicis-lycopersici* on 31 plant species from 10 botanical families by root dip method. They found that *Astragalus glycyphyllos*, *Phaseolus vulgaris*, spinach, beets and 10 tomato cultivars except Zuiken and Mato were highly susceptible. Brinjal, melon cv. Earl's knight natsu, faba beans, *Trifolium pratense* and *T. repens* were slightly susceptible. Root growth was poor and majority of the plants were discoloured. Eighteen cultivars from the Brassicaceae, Apiaceae, Asteraceae, Liliaceae and Poaceae were found symptomless.

Gulli and Chafai (1995) established the pathogenicity of *F. solani* on 2 and 6 months old seedlings of citrus in Morocco. They found that only 2 month old seedlings were susceptible to the fungus. A higher mortality rate was found when roots were

wounded before inoculation than when seedlings were transplanted directly in to soil infested with the pathogen.

Bankole (1996) isolated a total of 18 fungal species belonging to nine genera from the seeds of two tomato varieties viz., Ibadan local and Ife-1. Most of these fungi were present as surface contaminants, while the internally seed-borne fungi were *Al. longissima*, *As. flavus*, *As. niger*, *Cur. lunata*, *F. moniliforme*, *F. oxysporum* and *Phoma destructiva*. When the internally seedborne fungi were tested for their pathogenic potential on blotter, all the test fungi except *As. flavus* and *As. niger* exhibited varying degrees of pathogenic effect on two tomato varieties. The most pathogenic fungus on Ibadan local was identified as *F. oxysporum*, whereas *Ph. destructiva* was the most pathogenic on Ife-1. These fungi were more pathogenic on inoculated seeds, sown in sterilized soils than those sown in unsterilized soils.

Javed *et al.* (1997) tested the pathogenic potential of *F. solani* on seed germination of onion in the experimental dishes and pot conditions. They reported that *F. solani* reduced germination of onion seeds by 28 and 67% in experimental dishes and pots, respectively. Under artificial conditions both *in vitro* and *in vivo* *F. solani* caused 100 % seedling mortality.

El-Arabi and Abughanian (1998) tested the pathogenic effect of twenty isolates of *F. oxysporum* f. sp. *lycopersici* obtained from different part of Libya on 8 common tomato cultivars under green house conditions. They reported that all the tomato cultivars were found susceptible to all the test isolates of the fungus. However, the pathogenic potential varied with the individual cultivars.

Ramezani *et al.* (1999) studied the effect of *F. oxysporum* f. sp. *ciceri* on chickpea with different Pi viz., 0.25, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00 and 5.00 g mycelium/kg soil on chickpea var. Banda local under pot conditions. They reported that highest root infection was observed at 3.0 g mycelium/kg soil initial inoculum level.

Bost (2001) conducted the pathogenicity test of 6 isolates of *F. oxysporum* f. sp. *radicis-lycopersici* on susceptible cultivars of tomato. They reported that all the isolates were found pathogenic to all the cultivars except Floralina. Cultivar Conquest was found most susceptible.

Bao *et al.* (2002) surveyed wide range of geographic areas to study the variation in pathogenic potential of 43 strains of *F. oxysporum* and one strain of *F. solani* from tomato roots. They reported that out of 43 *F. oxysporum* strains, 21 were nonpathogenic to tomato, 20 were pathogenic, including 13 strains of *F. oxysporum* f. sp. *lycopersici* and 7 strains of *F. oxysporum* f. sp. *radicis-lycopersici*, and 2 were other formae speciales of the fungus.

Cai *et al.* (2003) determined the pathogenic potential of thirty-nine isolates of *F. oxysporum* f. sp. *lycopersici* on tomato growing fields in California. They reported that thirty six isolates were pathogenic and three were nonpathogenic to tomato.

Singh and Singh (2004) conducted a pathogenicity test of *Fusarium* spp. viz., *F. solani* and *F. oxysporum* on chilli in test tube and pot. They reported that *Fusarium* spp. develop infection both in test tube and potted seedlings. *F. solani* was more virulent as compared to *F. oxysporum*. Recovery percentage of *F. solani* was 76 and 66 from the test tube and potted seedlings, respectively. However, recovery percentage of *F. oxysporum* was 46 to 50%. Mixed inoculation of *F. solani* and *F. oxysporum* resulted cent percent infection of seedlings in test tubes and recovery percentage was 99%.

Sharma *et al.* (2005 a) determined the pathogenic potential of *F. oxysporum* at different Pi viz., 0.5, 1.0, 1.5, 2.0, 2.5, 5.0 and 10.0 ml spore suspension having 10^8 cfu/kg soil on the plant growth and yield of mungbean cv. ML-1108 under pot conditions. They reported that reduction in all growth and yield parameters was observed with corresponding increase in Pi. The highest reduction in plant growth and yield was observed at highest Pi. 100% root infection was also observed at highest inoculum level.

Kumar *et al.* (2007) studied the pathogenic and biochemical variability among the 11 isolates of *Fusarium udum* collected from pigeon pea fields from Uttar Pradesh, New Delhi and Hyderabad. They reported that wilt incidence ranged from 13 to 100%.

Sant *et al.* (2008) studied the pathogenic effect of *F. oxysporum* f. sp. *lycopersici* at different inoculum viz., 2.5, 5.0, 10.0, 15.0 and 20.0 g/plant on vitamin C content and root-rot on different tomato cultivars viz., Arka Vikas, Co-3, DVRT-1, DVRT-2 and Panjab Chhuhara under pot conditions. They reported that the highest reduction in vitamin C content and percent root-rot per plant was found in highest inoculum level in tomato cv. DVRT-1 followed by 15.0 g, 10.0 g, 5.0 g and 2.5 g, respectively.

Since De Condole (1815) first described the genus *Rhizoctonia*, more than 100 species have been described so far. These species are distinguished from one another primarily in respect to morphology and dimension of sclerotia and monilioid cells, and on the basis of pathogenicity. *Rhizoctonia* as a group of fungi among soil borne pathogens causes economic yield losses on their host plants. They exist in nature as strains differing from each other in characteristics such as cultural conditions and virulence. Distribution of pathogenic groups vary with respect to the distribution of their host plants, while that of saprophytic ones vary according to the vegetation and climatic factors (Muyolo *et al.*, 1993 a, 1993 b; Ogoshi, 1996).

R. solani is one of the most destructive species of *Rhizoctonia*, occurring globally and causing various maladies starting from seed decay, damping off, root and stem rots, wilt, canker, sheath blight and ear rot on more than 500 hosts including tomato (Ogoshi, 1985). *R. solani* primarily attacks below ground plant parts such as the hypocotyls, roots, suckers, runners, rhizomes, corms etc. but is also capable of infecting above ground plant parts. It has a remarkable capacity to remain in soil saprophytically in the absence of host plant (Parmeter, 1970). There are many reports available regarding the pathogenic effects of *R. solani* on a number of vegetable crops (Nakai and Ui, 1977; Stephens *et al.*, 1982; Haseeb, 1983; Gallo Llobet *et al.*, 1988; Otrysko and Bonville, 1992; Gilligan *et al.*, 1996; Karaca *et al.*, 2000; Botha *et al.*, 2003).

Tello *et al.* (1990) established the pathogenicity of 10 isolates of *R. solani* on cucumber cv. corona and melon under green house conditions. They reported that all the isolates of *R. solani* were pathogenic when inoculated at 16-18 true leaves stage of cucumber and melon plants.

Otrysko and Banville (1992) studied the effect of *R. solani* on the yield and quality of 10 cultivars of potato tubers. They observed a significant reduction in total yield and quality of tuber in plants inoculated with the fungus. They also reported a significant increase in the number of malformed and fissured as well as number of tubers with black scurf.

Hall and Sumner (1994) reported that *R. solani* caused seedling mortality to watermelon under field conditions.

Keinath (1995) studied the effect of different initial inoculum levels of *R. solani* on the growth of 15 days old seedlings of cabbage cv. Gaurmet and reported an inversely proportional relationship between initial inoculum level and the crop yield.

Gilligan *et al.* (1996) studied the effect of long crop rotations on the inoculum density of *R. solani* in field plots of potato and reported that soil borne inoculum of *R. solani* produced stem canker even in a 6 year crop rotation with non-host crops.

Khangura *et al.* (1999) tested the pathogenic potential of 112 isolates of *Rhizoctonia* isolated from canola growing areas of western Australia on canola, crucifer, leguminous and cereal crops. They found that most of the isolates were pathogenic to canola at varying degree. Isolate ZG5 is the most pathogenic to crucifers, mildly virulent to leguminous crops but nonpathogenic to cereal crops. However, isolate ZG1-1, found pathogenic to legumes and cereals and highly pathogenic to canola.

Karaca *et al.* (2000) determined the pathogenic effect of 229 *R. solani* isolates obtained from bean fields of Samsun province, Turkey. They reported that all the selected isolates were virulent at varying degrees to eight plants from different families. Isolate HAF 1-3 were found to be the most virulent isolate. Sugar beet was found most susceptible while corn and leek were found to be rather resistant. Isolate AG 5 was found highly to moderately virulent to bean cultivars while isolate Horoz was found to be the most susceptible to bean cultivars.

Botha *et al.* (2003) isolated *Rhizoctonia* species associated from black root rot disease of strawberries in the western Cape province of South Africa and assessed their pathogenic potential and relative virulence. Both binucleate and multinucleate types were recovered from diseased roots and identified as *R. fragariae* and *R. solani*, respectively. All *Rhizoctonia* isolates tested were pathogenic to strawberry cv. Tiobelle, but *R. solani* (AG 6) was the most virulent causing severe stunting of plants. *R. fragariae* (AG-A) and (AG-G) were not as virulent as *R. solani* but also caused stunting. *R. fragariae* AG-I was the least virulent, and did not cause stunting of the plants, however, it incited small, pale, spreading lesions on infected roots. This is the first species confirmation and AG type identification of *Rhizoctonia* taxa causing root rot of strawberries in South Africa.

Balali and Kowsari (2004) established the pathogenicity of different isolates of *R. solani* on bean plants. All the tested isolates of *R. solani* were pathogenic to bean.

However, each isolates differ in the manifestation of symptoms and the extent of disease severity.

Tyagi *et al.* (2005) studied the pathogenic potential of wilt fungus, *F. oxysporum* f. sp. *ciceri*, root rot fungi viz., *R. solani* and *M. phaseolina* at different Pi viz., 0.5, 1.0, 1.5 and 2.0 g mycelium mat/plant on plant growth and disease development on chickpea. They reported that the wilt/root rot disease development was increased and plant growth was decreased as the Pi increased. *F. oxysporum* f. sp. *ciceri* caused maximum damage to growth parameters of chickpea followed by *M. phaseolina* and *R. solani*.

Sarkar and Saxena (2007) studied the pathogenic potential of *As. niger*, *As. flavus*, *F. udum*, *Alternaria* spp., *Cur. Lunata*, *R. solani*, *M. phaseolina* and *Colletotrichum* sp. in terms of seedling infection and seed rotting of sunhamp under *in vitro*. They reported that *F. udum*, *R. solani*, *M. phaseolina* and *Colletotrichum* sp. showed a higher potential to cause seedling infection. *As. niger*, *As. flavus* and *Alternaria* spp. were mostly responsible for seed rotting. The potential loss due to the mycoflora varied in agar tube (9-18%) and starilized sand method (9-41%).

Plant parasitic nematodes are pathogens in their own right and are capable of producing recognizable disease symptoms on an appropriate susceptible host. Most of the diseases caused by nematodes are debilitating. However, when they interact with other pathogenic organisms the disease picture drastically altered. It changes from debilitating to annihilating (Powell, 1963, 1971 b, 1979; Bergeson, 1972). The varieties bred resistant against fungal attack become susceptible in the presence of nematodes (Atkinson, 1892; Neal, 1954; Melandez and Powell, 1965, 1967; Porter and Powell, 1967; Sumner and Johnson, 1973).

The literature on nematode-fungal interactions has been reviewed by Pitcher (1963, 1965), Miller (1965), Powell (1971a, 1979), Bergeson (1972), Norton (1978), Lamberti and Taylor (1979), Haseeb (1983), Webster (1985), Mai and Abawi (1987), Sasser (1989), Khan (1993), Evans and Haydock (1993), Back *et al.* (2002), Luc *et al.* (2002) etc. A vast majority of nematode interactions involve fungal pathogens, especially wilt and root-rot fungi. Of all the interactions with plant parasitic nematodes, none are more damaging to crops worldwide as the combined effects of wilt-inducing fungi and root-knot nematodes.

Atkinson (1892) for the first time reported that the infection by root-knot nematodes considerably increased the incidence and severity of Fusarium wilt in cotton. Since, then number of workers have made their efforts to work on the above subject.

Choo *et al.* (1990 a) studied the influence of *M. incognita* on the development of cucumber wilt by *F. oxysporum* f. sp. *cucumerinum*. They reported that the wilt was much more severe in plants inoculated with the nematode and fungus simultaneously than with the fungus alone. Plant growth was significantly reduced in plants inoculated with the fungus alone, nematode alone or both the pathogens simultaneously. Plant height and shoot weight were reduced more in plants inoculated with fungus and nematode simultaneously than by either pathogen alone. Root weight showed no statistical difference. The number of propagules of *F. oxysporum* f. sp. *cucumerinum* detected from the root and stem was higher in plants inoculated simultaneously with fungus and nematode than with fungus alone.

Choo *et al.* (1990 b) reported that the incidence of damping-off disease and rate of emergence of seedlings varied with the host plants viz., cucumber, pepper and tomato inoculated with *M. incognita* and *R. solani*. The seedling emergence was severely reduced by co-inoculation with *M. incognita* and *R. solani* in cucumber and pepper. However, less severe infection was found in tomato plants.

Khan and Husain (1991) studied the effect of *M. incognita* and *F. solani* on the growth of papaya under greenhouse conditions. They reported that *M. incognita* alone caused greater suppression of growth of papaya than *F. solani*. However, highest reduction in plant growth was observed in plant inoculated with both the pathogens simultaneously followed by the sequential inoculation of nematode 15 days prior to fungus and fungus 15 days prior to nematode, respectively. *F. solani*, whether inoculated simultaneously or sequentially, reduced the rate of reproduction of nematode but increased the rate of root rotting.

Singh *et al.* (1991) determined the effect of *M. incognita* and *R. solani* alone and in combination on tomato cv. Perfection. Results indicated that the highest reduction in plant growth was observed in plants inoculated with both the pathogens simultaneously.

Ali and Venugopal (1992) studied the interactive effect of *M. incognita* and *R. solani* on disease development of cardamom seedlings. They reported that damping off or

rhizome rot disease was more severe in combined inoculation than in either pathogen alone. *M. incognita* predisposed the seedlings to *R. solani*.

Shah *et al.* (1993) studied the individual and concomitant effects of *R. solani*, *As. niger* and *M. javanica* on the growth of chilli and reproduction of nematode in a pot experiment. All the pathogens reduced plant growth significantly when inoculated alone. However, maximum reduction in plant growth was observed in plants inoculated with *R. solani* and least with *As. niger*. Reduction in growth parameters was further increased in simultaneous inoculation with *R. solani* and *M. javanica*.

Walia and Gupta (1994) studied the interactive effects of *R. solani* and *M. javanica* on tomato. They reported that there was a reduction in number of galls, when the fungus and nematode were inoculated simultaneously. However, highest root necrosis and reduction in plant growth was observed in plants inoculated with nematode 7 days after fungus.

Kassab and Ali (1995) studied the effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato cv. Walter, resistant to fungus. They observed that pre-inoculation with nematodes allowed fungus to readily and more extensively colonize the root than in plants inoculated with both the pathogens simultaneously or when the fungus preceded nematodes.

Makhnotra *et al.* (1997) studied the interaction of *M. incognita* with *F. oxysporum* in rhizome rot of ginger under pot conditions. They revealed that disease incidence was higher in plants inoculated with nematode and fungus simultaneously, than in plants inoculated with either pathogen singly. The nematode was found to be more damaging to plant growth than the fungus alone.

Abdel-Momen and Starr (1998) studied the interaction of *M. javanica* and *R. solani* on peanut in greenhouse and field microplot experiments. The effects of *R. solani* on reproduction of *M. javanica* was variable with nematode Pi having a greater effect on nematode reproduction than did the presence of *R. solani*. In microplot tests peanut pod rot and root colonization by *R. solani* were increased by the presence of *M. javanica* and the total amounts of pod rot and root colonization were positively related to Pi of *M. javanica*. Pod yield was more suppressed by both pathogens than by either pathogen

alone, and was negatively related to Pi of *M. javanica* in both microplot experiments. The data confirm the interaction of *M. javanica* and *R. solani* on peanut.

Jonathan and Rajendran (1998) studied the interaction between *M. incognita* and *F. oxysporum* f. sp. *cubense* on banana cv. Rasthali. Synergistic interaction occurred between the pathogens both in concomitant and sequential inoculations, resulting in significant reduction in plant growth. The Panama wilt disease in terms of corm rot was significantly higher when prior inoculation of nematode was done followed by the fungus alone and concomitant inoculations of both the pathogens respectively.

Imran (1999) investigated the effect of *M. incognita* on the development of tomato wilt caused by *F. solani*. The growth of the plants were reduced with the inoculation with either of the pathogen alone. The inhibition of growth of the plants were highest when fungus and nematodes were inoculated simultaneously, followed by nematode inoculation prior to fungus. The least damage to the plant was observed when fungus inoculation was made prior to the nematode.

Bhagwati and Goswami (2000) studied the interaction of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato. Synergistic effect was recorded in treatments with either simultaneous inoculation of both the pathogens or when nematodes were inoculated prior to fungus. Greater damage was recorded in plants inoculated with both the pathogens simultaneously than those inoculated with *Fusarium* alone.

Singh and Goswami (2001) studied the interactive effect of *M. incognita* and *F. oxysporum* on cowpea cv. Pusa Komal under pot conditions. They reported that wilting was increased when plants were inoculated with *M. incognita* in combination with *F. oxysporum* as compared with *F. oxysporum* alone inoculated plants. Nematode inoculation preceded by fungal inoculation caused more wilting than plants inoculated with both the pathogens simultaneously.

Chand and Tripathi (2002) determined the effect of *M. incognita* (100 and 1000 J₂/kg soil) and *R. solani* (1.0, 1.5 and 2.0 g mycelium/kg soil) on the root rot incidence of tomato cv. Hisar Arun. They found the synergistic effect of *R. solani* and *M. incognita* in causing root rot of tomato. Inoculation of 1.0 g fresh mycelium of *R. solani* and 100 J₂ of *M. incognita* resulted in 55.59% disease incidence. The disease incidence was increased

up to 98.80% when seedlings were inoculated with 2.0 g fresh mycelium of fungus and 1000 J₂/kg soil.

Pathak and Keshari (2004) studied the interaction of *M. incognita* with *F. oxysporum* f. sp. *conglutinans* on cauliflower under pot conditions. They revealed that wilt appeared only in those treatments where *F. oxysporum* was inoculated either singly or in combinations with *M. incognita*. Inoculation of only nematodes did not produce wilting in cauliflower plants.

Roy and Mukhopadhyay (2004) determined the individual and combined effect of *M. phaseolina* and *M. incognita* on brinjal cv. Makra under greenhouse conditions. They reported that the inoculation with *M. phaseolina* alone and in combination with *M. incognita* significantly reduced the plant height by 59 and 64 % respectively as compared to the uninoculated control. Plant mortality was 100% in plants inoculated with nematode and fungus simultaneously, whereas reduction in plant mortality was 66.7% and 24% in plants inoculated with fungus alone and nematode alone, respectively.

Akram and Khan (2006) studied the interactive effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on the plant growth and yield of tomato using sequentially and concomitantly inoculations under field conditions. They reported that the nematode and fungus acting alone caused root galls and fusarial wilt, respectively and reduced the plant growth and yield. Fusarial wilt is become considerably severe in the presence of root-knot nematode, especially in pre (38.0%) or concomitant (14.3%) inoculation. Highest reduction (41.8%) in fruit yield was observed in nematode prior to fungus followed by concomitant treatment (33.3%).

Samuthiravalli and Sivakumar (2008) studied the interactive effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato cv. CO-3 under glass house conditions. They reported that the effect of nematode in combination with the fungus enhanced the suppression of plant growth than that of the fungus alone. Highest reduction in plant height (33.08 cm) was observed in nematode fungus simultaneously inoculated plants.

Pest management can be defined as a practice whereby population of phytopathogenic microorganisms are maintained at levels that do not inflict economic

losses. Several methods have been in practice for the management of pathogen, which include chemical and non-chemical.

Chemical methods include the use of fumigant and non-fumigant nematicides for the control of plant parasitic nematodes (Taylor, 1975; Wright, 1981; Johnson, 1985; Johnson and Feldmesser, 1987).

Historically, one of the first major efforts to manage plant parasitic nematodes was made in 1881 in Germany in sugarbeet industry, later on a breakthrough was achieved in 1943 and 1945 with the discoveries of the potential of DD and EDB, respectively as effective soil fumigants (Thorne, 1961). Non-fumigant chemicals were tested under field conditions for their nematicidal activity during the late 50's and early 60's. These materials were usually carbamates or organophosphate and most, if not all of them were first tested for their insecticidal qualities and subsequently, their nematicidal activity was also investigated. Although numerous chemicals have been tested for nematicidal properties, very few have been developed and even fewer are currently used in managing plant parasitic nematodes (Heald, 1987). Carbofuran is one of the most widely used nematicide comes under carbamate group which is available as granules or liquids of low volatility. It is easily soluble in water and systemic in nature and acts by inhibiting the enzyme cholinesterase and causing paralysis and death of affected nematodes and insects (Agrios, 2005).

Kaul and Chhabra (1990) determined the efficacy of phorate and carbofuran either at 1.5 g/plant as spot treatment or in 2 split doses of 0.75 g/plant at the time of transplanting and 30 days after transplanting as basal dressing to field of brinjal infested with *M. incognita*. All the nematicidal application increases the yield significantly. Split doses further enhanced the yield over single applications in both the cases. Carbofuran was found more effective than phorate in reducing yield losses. Nematode populations were also lower in roots and soil having split doses.

Babatola and Omotade (1991) evaluated the effect of carbofuran (3 and 5 kg a.i./ha) and ethoprop (2 and 4 kg a.i./ha) in a field trial during 1987 and 1988 for the control of *P. brachyurus* and *M. incognita* on cowpea. Nematode populations in both soil and roots of cowpea were reduced significantly by treatments with nematicides. Number of pods per plant, average number of grains per pod, weight of 100 grains and grain

yields were significantly improved by the treatments. Root gall indices were decreased significantly by all the treatments.

Poornima and Vadivelu (1993) determined the effect of various nematicides viz., carbofuran, phenamiphos, and phorate on *M. incognita*, *P. delattrei* and *R. reniformis* infesting brinjal cultivar CO-2. They found that phenamiphos was the best in enhancing the yield and growth of brinjal followed by carbofuran and phorate, respectively.

Amin *et al.* (1994) studied the effect of trifluralin @ 3.5 l/ha, aldicarb, oxamyl, carbofuran, diazinon each @ 30 kg/ha or sulphur @ 40 kg/ha for the management of *M. incognita* infesting tomato in green house conditions. They found that aldicarb gave highest reduction in population of *M. incognita* in roots followed by oxamyl, sulphur and trifluralin, respectively. Whereas, treatment with carbofuran and diazinon, resulted in the increase of nematode population in roots. Highest yield of tomato was found with the treatment of aldicarb.

Sharma and Khan (1995) determined the efficacy of carbofuran, ethoprophos, phorate, ebufos and benfuracarb @ 6 and 3 kg a.i./ha for the management of *M. incognita* infesting tomato cv. Moneymaker under field conditions. They reported that the phorate gave maximum percent reductions in root gall index (52.6 and 42.1 at 6 and 3 kg a.i./ha, respectively) and maximum percent increase in yield (65.2 and 38.5 at 6 and 3 kg a.i./ha, respectively) while benfuracarb was found to be least effective in increasing the yield.

Joshi and Patel (1996) studied the effect of carbofuran, carbosulfan and quinolphos @ 0.5, 1.0 and 1.5% (w/w) as seed treatment and carbofuran, cadusafos, HOE 388, phenamiphos, phorate and quinolphos @ 1, 2 and 3 kg a.i./ha as soil treatment against 1000 J₂ of *M. javanica*/kg soil on groundnut under pot conditions. They reported that seed treatment with carbosulfan and quinolphos both @ 1.5% (w/w) reduced the nematode penetration in roots and significantly improved the plant growth. While among the soil treatments, phorate @ 3 kg a.i./ha was found effective followed by carbofuran in improving plant growth.

Siddiqui *et al.* (1998) evaluated root dip treatment with carbofuran and fenamiphos for the management of *M. incognita* and yield of tomato and brinjal. They reported that the highest increase in yield of both the crops was found in seedlings treated with fenamiphos @ 0.3 g a.i./100 ml water. The root gall formation on tomato was

significantly reduced when seedlings were treated with both carbofuran and fenamiphos @ 0.3 g a.i./100 ml water.

Yadav and Mathur (1999) conducted field experiments at 2 sites infected with *M. incognita* (2 J₂/g soil) for two years, to study the percent avoidable loss in yield of green chillies cv. Jaipur local with spot application with carbofuran/phorate @ 2 kg a.i./ha. Studies indicated that average percent avoidable yield loss of 53.36 and 39.76 was found in carbofuran and phorate treated plots respectively.

Vadhera *et al.* (2000) evaluated the efficacy of nursery treatment and seedlings root-dip application of some nematicides for the management of *M. incognita* on tomato cv. Pusa Ruby under field conditions. They observed that carbofuran @ 0.6 g a.i./m² in a nursery bed treatment improved seed germination and seedlings vigour and significantly reduced the gall index of tomato from 5 to 3.6 and increased the yield of tomato by 36.9%. Seedlings root dip for 6 hrs in carbosulphan and triazophos @ 0.1% increased yield by 43 and 42% respectively and reduced gall index to 3.5 and 3.8 respectively as compared to 5.0 in the control.

Tiwari *et al.* (2002) studied the effect of carbofuran and dazomet @ 0.3 and 0.6 g a.i./m² each against *M. incognita* on tomato in nursery beds. They revealed that the maximum yield (362 q/ha) was recorded in carbofuran (@ 0.6 g a.i./m²) treated nursery beds followed by dazomet (354 q/ha) (@ 0.6 g a.i./m²), respectively. However, 60% reduction in gall index was found in dazomet treated beds. Whereas, 55% reduction was found in carbofuran treated beds at the same concentration.

Hussain and Bora (2006) studied the comparative efficacy of nematicides *viz.*, triazophos and monocrotophos each @ 0.1% as seed soaking, phorate and carbofuran each @ 3.0 kg a.i./ha as soil treatment and neem cake @1500 kg/ha as soil treatment against *M. incognita* on french bean under pot conditions. They reported that highest reduction in root galls and egg masses was found in the treatment with phorate followed by triazophos, monocrotophos, carbofuran and neem cake, respectively.

Mhase and Pawar (2007) studied the effect of different doses (@ 1.5 and 3.0% w/w as seed dressing and 0.1 and 0.2% as seed soaking for four hours) of carbosulfan against *M. incognita* on mung under field conditions. They reported that seed dressing with carbosulfan @ 3.0% w/w were found to be the most effective in reducing the root-

knot nematode population (49.27%) and gall index (47.88%) and increasing the yield (52.32%) of mung with 1:1.49 cost benefit ratio.

Mashela *et al.* (2008) studied the effect of non-fumigant nematicides viz., aldicarb @ 150 g a.i./ha and fenamiphos @ 100 g a.i./ha against *M. incognita* on cucumber under field conditions. They reported that both the products reduced the number of nematodes in tomato roots (83-99%) and increased the fruit yield (72-94%) of tomato.

Management of nematodes through non-chemical methods and search for ecofriendly pesticides from plant origin is gaining importance now a days. (Sayre *et al.*, 1964; Singh and Sitaramaiah, 1973; Rodriguez-Kabana, 1986).

Amending the soil with farmyard manure and commonly available plant parts and products of neem, murraya, castor, mustard, linseed in the form of oil cakes; dry leaves, seeds, seed kernel, seed coat and seed powder etc. is one of the common methods used against plant parasitic nematodes, especially in India. Among various organic amendments used till date, plant parts and products of neem have been extensively used by number of workers for the management of plant parasitic nematodes, as they contain several nematicidal compounds (Singh and Sitaramaiah, 1967, 1970, 1971, 1973; Alam *et al.*, 1980; Haseeb, 1983; Rodriguez-Kabana, 1986; Alam, 1990; Mojumdar, 1995; Dash and Padhi, 1998; Singh *et al.*, 1999; Haseeb, 2003, 2004).

Singh *et al.* (1990) determined the effect of neem, castor and mustard cakes against *M. incognita* on tomato cv. Pusa Ruby under pot experiment. They reported that neem cake alone was highly effective against nematode followed by castor and mustard cakes, respectively. However, a mixture of neem + mustard and neem + castor cakes were more effective than neem cake only.

Alam (1991) studied the effect of mahua, castor, mustard, neem and groundnut oil cakes singly and in different combinations against *M. incognita*, *Trh. brassicae*, *R. reniformis*, *Hoplolaimus (Hop.) indicus*, *Hel. indicus* and *Tylenchus (Tyl.) filiformis* on tomato, brinjal, chilli, okra, cabbage and cauliflower under field conditions. He reported that all the treatments singly and in different combinations significantly reduced the population of plant parasitic nematodes. Mahua cake was phytotoxic to all the test crops except brinjal, whereas, other cakes improved plant growth significantly.

Mojumdar and Mishra (1993) studied the effect of neem cake, seed kernel/seed coat as single, full dose or split doses to soil naturally infested with *M. incognita* on chickpea under pot conditions. They found that all the treatments were effective to reduce the number of root galls significantly. However, treatment with neem seed kernels was most effective when applied as full dose.

Abid *et al.* (1995) studied the effect of neem dry leaves powder, seed powder and neem cake @ 2, 4 and 6 g/750 g soil against *M. javanica* on okra under pot conditions. They reported that all the treatments enhanced the plant growth and reduced gall formation as compared to untreated control. Maximum reduction in root-knot index was observed with oil cake followed by seed powder.

Rao and Goswami (1996) determined the efficacy of organic amendments viz., groundnut, karanj, mahua, mustard and neem cakes, an inorganic amendment, attapulgit based clay dust (ABCD) and carbofuran for comparison on root-knot development caused by *M. incognita* and growth of cowpea. They revealed that the reduction in root-knot development was significantly high in mustard, neem cakes, carbofuran and ABCD treatments, and the least effect being found with groundnut cake. The plant growth was greatly improved in mustard and neem cakes amended soil followed by karanj, mahua cakes and ABCD, respectively.

Akhtar and Mahamood (1997) determined the efficacy of suneem (Azadirachtin 80% a.i.) and neem oil for the management of *M. incognita* on tomato under pot conditions. They reported that seed treatments with suneem and neem oil significantly reduced the root-knot development and nematode population in roots. Suneem showed no phytotoxicity and improved plant growth, whereas treatment with neem oil showed significantly phytotoxic effect.

Dash and Padhi (1998) studied the comparative efficacy of four neem products eg. neem bitter @ 0.5%, repelin @ 1%, welgrow @1% and neem seed kernel extract @ 2.5% against 1000 J₂ of *M. incognita*/kg soil on tomato cv. Pusa Ruby by seed treatment, bare root dip of seedlings and foliar spray treatments. They reported that all the neem products effectively reduced the root-knot disease incidence and increased the plant growth. Neem bitter exhibited better performance in all the three methods of treatments.

Sharma *et al.* (2000) studied the effect of seed soaking by neemark and nimbecidine @ 1, 5 and 10% alone and also combined treatment of neemark and nimbecidine 1 and 5% with hostathion 1% for 2, 6 and 24 h against *M. incognita* infecting okra cv. Pusa Sawani. Significant reduction in number of galls was observed in treatment with neemark and nimbecidine 5% with 6 and 24 h soaking. Plant growth was improved with the increase in concentration of neemark and nimbecidine in 6 h soaking. A reduction of 10% in plant growth was recorded in treatment where seed soaking was done with nimbecidine for 24 h. Hostathion 1% alone was effective for 2, 6, and 24 h soaking in reducing the number of galls. Hostathion 1% in combination with neemark did not exhibit any complimentary effect in mitigating the nematode population or plant growth while with nimbecidine, some complimentary effect was observed.

Siddiqui and Alam (2001) evaluated the nematicidal effects of fresh floral parts, decomposed flowers, decomposed fruits (seeds), leaves and bark of neem and *Melia azedarach* @ 100 g each on tomato cv. Pusa Ruby inoculated with 5000 J₂ of *M. incognita*/kg soil. The root-knot development on tomato was significantly inhibited by all the treatments after three months of treatment. The lowest root-knot index was recorded in plants grown in soil amended with the decomposed seeds of both the species. The growth of tomato was also significantly improved by all the treatments. However, treatment with neem was more effective than *M. azedarach*. Further, decomposed material was more effective than fresh floral parts in controlling nematodes and increasing plant growth.

Oka and Yermiyahu (2002) studied the suppressive effects of two composts viz., cattle manure and grape marc against *M. javanica* infesting tomato under pot and *in vitro* conditions. They found that no root galls were found on tomato roots grown in soils containing 10 or 25% (v/v) cattle manure, and very few on those grown in 50% grape marc. Significant reductions in gall index were also found on tomato plants grown in soil containing lower concentrations of these compost. Water extract of the cattle manure showed high nematicidal activity to the nematode juveniles and less activity towards the eggs under *in vitro* test. However, water extract of the grape marc showed less nematicidal activity to the juveniles and eggs.

Sahoo *et al.* (2004) studied the comparative efficacy of different locally available organic/inorganic materials *viz.*, fresh leaves of acacia, eucalyptus and neem each @ 5 t/ha, neem cake @ 2 t/ha, lime, silkworm litter, simaruba seed powder each @ 2.5 t/ha and carbofuran @ 2.0 kg a.i./ha as a check for the management of *M. incognita* in okra under field conditions. They reported that all the treatments significantly reduced the root-knot index and improved the yield. Carbofuran recorded lowest (1.6) root-knot index, which was at par with the application of neem cake (1.6) closely followed by the application of fresh neem leaves (1.6) and silkworm litter (1.7). Highest yield (64.0 q/ha) was also obtained from carbofuran treated plots, which was at par with neem cake (62.0 q/ha) and neem leaves (61.0 q/ha).

Rajendran and Saritha (2005) studied the effect of *Arnica montata*, *Calendula officinalis*, *Carica papaya* and *A. indica* plant extracts against *M. incognita* infesting tomato cv. PKM-1. They reported that all the plant extracts reduced the root galls and nematode population in soil. Maximum mortality was recorded in plants treated with *A. indica*.

Yadav *et al.* (2006 b) studied the effect of oil cakes *viz.*, karanj, neem, mustard, castor and mahua as soil application for the management of *M. incognita* on chickpea under pot conditions. They reported that neem and karanj cakes were most effective in improving the plant growth and decreasing the nematode reproduction.

Rather and Siddiqui (2007) studied the effect of neem cake, neem leaves and neem gold (each @ 25 and 50 g/kg soil) against *M. incognita* @ 2000 J₂/kg soil in tomato cv. Saimla under pot conditions. They found that lower dose of each organic amendments is more effective than higher dose. Neem cake @ 25 g/kg soil was found most effective in reducing the root-knot infection and improving the plant growth followed by the same dose of neem gold and neem leaves.

Singh *et al.* (2008) studied the effect of organic amendments *viz.*, neem gold and neem seed kernel extracts (each @ 100 ml/kg seed v/w) against *M. incognita* on green gram. They reported that neem gold was found most effective in increasing the plant growth and reducing the root galls, egg masses and nematode population (85.45%, 63.9% and 42.1%, respectively) followed by neem seed kernel extract, respectively.

Biological control of plant pathogens is a distinct possibility for future and it can be successfully exploited in modern agriculture. The discovery of new biocontrol agents and demonstration of their impact in reducing disease incidence and severity has opened new avenues for practical applications in agriculture and for promoting environmental safety (Boland, 1990). During the past three decades, the research on biological control of nematodes has been the subject of interest all over the world (Mankau, 1980 a, b, 1981; Tribe, 1980; Jatala *et al.*, 1980; Rodriguez-Kabana *et al.*, 1984; Kerry, 1990; Stephan *et al.*, 1998; Meyer *et al.*, 2001; Sharon *et al.*, 2001; Haseeb, 2003, 2004; Siddiqui and Shaukat, 2004). Among various biocontrol agents, *Trichoderma* spp. is one of the most widely used fungal antagonist, which is also reported to have inhibitory effect to nematodes especially *Meloidogyne* spp. (Windham *et al.*, 1989; Sharon *et al.*, 2001; Siddiqui *et al.*, 2001; Haseeb, 2003, 2004; Zaidi *et al.*, 2004).

Khan and Saxena (1997) determined the effect of root dip treatment with culture filtrates of *As. niger*, *Paecilomyces lilacinus* and *T. viride* on multiplication of *M. javanica* and yield of tomato and reported that culture filtrate of all the bioagents significantly reduced the root-knot development and improve the yield of tomato under pot experiments.

El-Moity *et al.* (1998) conducted an experiment to determine the effect of three antagonists, *T. harzianum*, *Gliocladium virens* and *Bacillus* sp. (B.15) alone or in combinations against *M. incognita* on tomato plants. The treatments were applied once (one week before transplanting) or twice (before planting and 20 days after planting) @ 20 ml/2.5 kg soil. Studies indicated that the treatment with *Bacillus* sp. (B.15) was found to be the best for the management of *M. incognita*. Whereas, the least effective treatment was *T. harzianum*. No synergistic effect was observed when these antagonists were applied in different combinations. Application of the antagonist to infested soil twice was more effective than with single treatment. Treatment with the antagonists separately or in combination decreased the number of egg masses, eggs per plant and J₂ in soil, and increased the fresh weight of plants.

Khan *et al.* (2000) determined the comparative efficacy of chemical viz., tenekil and biocontrol agents viz., *P. lilacinus* and *T. harzianum* for the management of *M. incognita* on tomato. They reported that treatment with *P. lilacinus* and *T. harzianum*

when applied with organic substrate was found highly effective in decreasing the number of galls (110.4) per plant followed by tenekil (123.4) as compared to control (375.60).

Siddiqui *et al.* (2001) studied the effect of culture filtrate of *T. viride*, *T. harzianum*, *T. koningii* and *T. pseudokoningii* for the control of *M. javanica* in okra and mungbean. They reported that culture filtrate of all the biocontrol agents significantly reduced egg hatching and showed nematicidal activity by killing J₂ of *M. javanica* under *in vitro* conditions. Soil application of *T. harzianum* @ 25 ml/pot significantly reduced nematode population densities and root-knot development and increase the plant growth of both the plants. Highest reduction in nematode population in soil was also found in *T. harzianum* treated plants.

Devi and Sharma (2002) studied the effect of *T. viride* and *T. harzianum* @ 1 g/kg soil against *M. incognita* (500 J₂/kg soil) on tomato. They reported that both the treatments improved the plant growth and reduced the nematode population. However, variation among the treatments was not significant.

Goswami and Mittal (2004) studied the effect of *T. viride* and *P. lilacinus* (each @ 1 g fungal mat containing 2×10^6 cfu/500 g soil) alone and in combination for the management of *M. incognita* (@ 1000 J₂/500 g soil) under *in vitro* and pot conditions. They reported that culture filtrates of *T. viride* and *P. lilacinus* showed larvicidal effect and also parasitized the egg masses of *M. incognita* under *in vitro* conditions. Both the bioagents reduced the nematode population in roots and soil in both the treatments i.e., singly and concomitantly. However, both the fungi when applied in combination, the plant growth was increased significantly.

Yadav *et al.* (2005) studied the effect of biocontrol agents viz., *T. viride*, *As. niger* and neem seed powder against root-knot disease of brinjal. They reported that highest germination was recorded with *T. viride* treated seeds followed by *As. niger* and neem seed powder, respectively. Highest reduction in number of galls per root system was also found in *T. viride* treated seeds followed by *As. niger* and neem seed powder, respectively.

Sharma *et al.* (2006) studied the effect of two biocontrol agents viz., *P. fluorescens* (@ 4, 8, 16 g/kg seeds) and *T. viride* (@ 2, 4 and 8 g/kg seeds) and two nematicides viz., phorate and carbofuran (each @ 1, 2 and 4% w/w) as seed dressers

against *M. incognita* on chilli in nursery. They reported that the antagonists and nematicides at their highest dose of application were more effective in reducing root-knot index and nematode population.

Several potential bacterial antagonists were also reported for the management of *Meloidogyne* spp. Number of attempts have also been made to identify bacteria with most interest being centered on rhizosphere inhibiting bacteria. Plant growth promoting rhizobacterium, *Pseudomonas fluorescens* have emerged as one of the promising group of bacteria, which are mostly antagonistic to *Meloidogyne* spp. (Gokte and Swarup, 1988; Oostendrop and Sikora, 1989; Shanthi and Sivakumar, 1995; Haseeb, 2003; 2004; Zaidi *et al.*, 2004).

Hoffmann-Hergarten *et al.* (1998) found the biocontrol activity of three out of 15 bacterial strains pre-selected for antagonistic activity in different pathosystems against *M. incognita* on lettuce and tomato. Seed treatment with the rhizobacteria *Pseudomonas* sp. W34 or *B. cereus* S18 resulted in significant reductions in root galling and enhanced seedling biomass. Significant reduction in *M. incognita* gall index was observed within 18 weeks after inoculation with all the 3 bacterial strains i.e., *Pseudomonas* spp. W34, *B. cereus* S18 and *B. subtilis* VMI32. *B. cereus* S18 caused 9% yield increase when compared with the nematode control and thereby compensated the yield loss due to nematode infection.

Hanna *et al.* (1999) determined the efficacy of *B. thuringiensis*, *B. subtilis* and *P. fluorescens* each @ 150 ml containing 10^8 cfu/ml for the control of *M. incognita* on tomato using bioassays and greenhouse tests. Both *B. thuringiensis* and *P. fluorescens* isolates showed the highest nematicidal activity against hatched juveniles and adults of *M. incognita*. The mortality levels of juveniles and adults of *M. incognita* increased with the highest concentrations of all the three bioagents (10^5 , 10^8 and 5×10^8 cfu/ml). Generally, the percentage of root-knot development were decreased when the antagonistic bacteria were introduced prior to nematodes compared to the simultaneous inoculation of both the nematode and bacteria.

Jonathan *et al.* (2000) determined the effect of *B. cereus*, *B. subtilis*, *B. sphaericus*, *Agrobacterium radiobactor*, *P. fluorescens*, *P. chlororaphis* and *Burkholderia cepacia* (@ 20 ml/pot containing 2×10^9 cfu/ml each), uncharacterized

actinomycetes (strains 29 and 45) and the nematode-parasitic bacterium, *Pasturia penetrans* (isolate 100) (@ 5 g/pot) for the management of *M. incognita* race-1 (@10,000 eggs/pot) on tomato and banana under green house conditions. All the bacteria enhanced the growth of both crops and suppressed root-gall development on tomato as compared to control plants. Root gall indices on tomato inoculated with *M. incognita* and bacteria ranged from 25 to 31% as against 94% in the nematode alone inoculated plants. The bacteria also limited reproduction of the nematode on both crops i.e. reproduction factor ranged from 9-24 in bacteria treated plants as against 143 in nematode alone treated plants.

Siddiqui *et al.* (2002) determined the effect of *P. fluorescens* strains GRP3 and PRS9, *Azospirillum brasilense*, *Azotobacter chroococcum* and the commercial "Microphos culture" (a mixture of *P. striata* + *B. polymyxa*) and the fungus *As. awamori* @ 1 g/kg soil against *M. incognita* on brinjal under green house conditions. They reported that the Microphos culture was better in improving plant growth and reducing root-knot development and nematode multiplication than the other biocontrol agents. *P. fluorescens* strain GRP3 was found better for the management of *M. incognita* than strain PRS9. Whereas, *A. brasilense* was least effective in improving plant growth and reducing nematode multiplication. Best management of *M. incognita* was obtained when the Microphos culture was used with *A. chroococcum* and *A. brasilense*.

Jothi *et al.* (2003) conducted a field trial to study the effect of *P. fluorescens* (20 g/m² having 1.5×10^8 cfu/g) against *M. incognita* in tomato nursery and reported that *P. fluorescens* treated plants gave maximum yield (64.3%) and minimum soil population of the nematode (56% reduction). The plant height was also highest in *P. fluorescens* treated plots. The soil population of the nematode and gall index were reduced significantly.

Siddiqui and Shaukat (2004) determined the influence of *T. harzianum* strain Th-6 on the biocontrol performance of *P. fluorescens* strain CHA0 and its derivative CHA0/pME3424 against *M. javanica* infesting tomato. They reported that *T. harzianum* improved biocontrol of *M. javanica* by *P. fluorescens* in tomato under *in vitro* and glass house conditions. They also observed that when *P. fluorescens* and *T. harzianum* applied together in unsterilized sandy loam soil caused greater reduction in nematode population densities in tomato roots.

Mohanty *et al.* (2007) studied the effect of *P. fluorescens* @ 20 g/m² against *M. incognita* on brinjal in nursery under sick field conditions. They reported that seedlings transplanted from treated nursery to the field gave 28.7% increase in yield and also decreasing the root-knot population.

Management of plant parasitic nematodes through various methods have been tried independently world over since last century. Largely chemicals are being used in huge quantities for combating nematode diseases. During last five decades, it had exerted serious impact on non-target and beneficial soil microflora and fauna as well as toxicity to animals and human beings, which compels us to develop alternative nematode management strategies. In recent past, attempts have been made to develop integrated means for the management of nematode pest (Gaur and Prasad, 1986; Jayaraj *et al.*, 1993; Philip, 1993; Gautam *et al.*, 1995; Haseeb, 2003).

Jain and Bhatti (1991) determined the comparative efficacy of aldicarb @ 1.0 kg a.i./ha and neem leaves @ 50 q/ha for the control of *M. javanica* infecting tomato cv. HS-101. They reported that reduction of 48.9% and 26.7% nematode population was obtained in nursery treated with neem leaves + spot application of aldicarb and nursery treated with neem leaves + spot application of neem leaves respectively. Aldicarb treated nursery seedlings with spot application of aldicarb at transplanting showed maximum reduction (62.3%) in nematode population. However, uninfected tomato nursery + aldicarb treatment gave highest yield of 7.6 kg/plot (2.0 x 1.5 m²) in comparison to 4.7 kg in plot transplanted with infected tomato nursery without any treatment.

Mohanty *et al.* (1995) revealed that pre-planting application of neem cake @ 1 t/ha followed by application of carbofuran @ 1 kg a.i./ha 45 days after planting gave the best result in terms of suppression of *M. incognita*, disease intensity and increased yield of ginger.

Barman and Das (1996) studied the effect of carbofuran @ 3% (w/w) and organic amendments *viz.*, neem, mustard cakes and poultry manure each @ 2 t/ha alone and in combination for the management of *M. incognita* (265 J₂/200 g soil) on green gram under field conditions. They reported that all the treatments alone and in combinations were found effective in improving plant growth, yield, and in reducing the number of galls, egg masses and final nematode population of *M. incognita* as compared to untreated

control. However, poultry manure @ 2 t/ha was found highly effective followed by the combined treatment with carbofuran @ 3% (w/w) as seed dressing + neem cake @ 1 t/ha.

Rao *et al* (1997) studied the effect of neem cake and *T. harzianum* alone and in combination for the management of *M. incognita* on tomato. They found significant increase in the plant growth and reduction in root galling and final population of *M. incognita* on tomato seedlings grown in neem cake amended soil and treated with *T. harzianum*. They also observed that colonization of *T. harzianum* on tomato increases in the above treatments.

Siddiqui *et al* (1999) determined the effect of neem cake and brown seaweeds viz., *Stoechospermum marginatum* and *Sargassum tenerrimum* (each @ 1% w/w) with or without *P. aeruginosa* (@ 200 ml/pot containing 2.4×10^8 cfu/ml) for the management of *M. javanica* on tomato. They reported that ethanolic extract of neem cake and brown seaweeds viz. *S. marginatum* and *S. tenerrimum* caused significant mortality of *M. javanica* juveniles under *in vitro* conditions. Soil amendment with neem cake and brown sea weed alone or mixed with *P. aeruginosa*, significantly reduced number of galls, egg mass production and population of *M. javanica* in soil. Use of organic amendments separately or mixed with bacterium significantly increased biomass of tomato foliage and no adverse effect of organic amendments was observed on bacterial survival in the rhizosphere.

Khan (2000) studied the management of *M. incognita* in tomato nursery through *P. fluorescens* @ 20 g/m² and carbofuran @ 1.5 g/m² both separately and in combination under field conditions. He reported that *P. fluorescens* when applied separately, the seed germination was 87% and 71.4% as against 50% and 53.5% in control respectively in the year 1999 and 2000. The maximum yield and minimum gall index was recorded in *P. fluorescens* and carbofuran treated plots followed by carbofuran alone and *P. fluorescens* alone, respectively.

Srivastava (2000) conducted an experiment to develop integrated management of *M. incognita* infecting brinjal cv. Rajendra baigan II under field conditions. He reported that 15 days solarization during summer (May) with sub treatments viz., neem cake, poultry manure and farmyard manure each @ 200 g/m² and carbofuran @ 0.3 g a.i./m² was found significantly superior in respect of reducing root-knot index and increasing

yield over control. Gall index was reduced from 3.6 to 1.0 in solarized plots treated with carbofuran and neem cake as compared with unsolarized untreated plots. Yield was also significantly improved in comparison to untreated control.

Faruk *et al.* (2002) determined the efficacy of soil treatment with different isolates of *T. harzianum* (W-108, W-120, TG, TK, T33, and TV-1), mustard oil cake and poultry refuse against *M. incognita* and *M. javanica* on tomato under pot and field conditions. They found that all the treatments reduced severity of root-knot development and increased plant growth and fruit yield over the untreated control. However, efficacy of organic additives was improved significantly when these additives were applied with different isolates of *T. harzianum*. The soil treatment with isolate W-108 + mustard oilcake (MOC), W-108 + poultry refuse (PR), TV-1 + MOC, TV-1 + PR and TG + PR drastically reduced the severity of root-knot development and increased plant growth and the fruit yield of tomato.

Mahapatra and Mohanty (2003) conducted nursery and field experiments to determine the efficacy of *P. fluorescens* @ 10 and 20 g/m² alone or in combinations with carbofuran @ 1.5 g/m² against *M. incognita* infesting brinjal cv. Pusa Purple Long. They found that treatment with carbofuran had the highest reduction (46.4%) in root-knot index at transplanting. Whereas, treatment with *P. fluorescens* @ 20 g/m² and @ 10 g/m² + carbofuran caused 38.4 and 31.0% reduction in root-knot development, respectively.

Srivastava (2003) conducted an experiment for the development of integrated management of *M. incognita* infesting brinjal. He reported that solarization with sub treatments with neem cake, poultry manure and farmyard manure @ 200 g/m² and carbofuran @ 0.3 g a.i./m² significantly reduced root-knot development and increased the yield over unsolarized, untreated control. Gall index was reduced significantly (1.16) in solarized plots treated with carbofuran followed by neem cake (1.3), poultry manures (2.1) and farmyard manure (2.8) than unsolarized, untreated control plots (3.8).

Pankaj *et al.* (2004) studied the effect of neem seed powder and carbofuran alone and in combination against plant parasitic nematodes mainly *M. incognita* and *R. reniformis* on tomato under field conditions. The maximum reduction in the nematodes (*M. incognita* and *R. reniformis*) population was observed in carbofuran (83.3 and 75%)

followed by neem seed powder and carbofuran (66.7 and 62.5%), respectively. Neem seed powder alone was not as effective as when applied along with carbofuran.

Senthilkumar and Ramakrishnan (2004) determined the efficacy of *P. fluorescens*, *T. viride* and carbofuran singly and in combination for the management of *M. incognita* in okra cv. CO 3 under green house conditions. The application of *P. fluorescens* (2.5 kg/ha) singly and in combination with carbofuran significantly improved plant growth. Plants treated with *P. fluorescens* (1.25 kg/ha) + carbofuran (0.5 kg a.i./ha) recorded the highest fruit weight per plant. The highest number of colony forming units of *P. fluorescens* and *T. viride* was obtained in separate application of each of these treatments compared to combined applications.

Tariq and Siddiqui (2005) studied the effect of neem leaves, neem oil cake @ 50 g/kg soil and carbofuran @ 0.1 g a.i./kg soil alone and in combination against 5000 J₂ of *M. incognita*/kg soil on tomato cv. Pusa Ruby. They found that highest inhibition in penetration of nematode juveniles was observed in neem cake extract with carbofuran as root dip treatment for 120 min. Highest reduction in root-knot development and highest improvement in growth was observed in plants treated with neem cake and carbofuran.

Kumar and Khanna (2006) studied the effect of *T. harzianum* and neem cake alone and in combination against root-knot nematode on tomato under pot conditions. They reported that the fungus was highly effective against *M. incognita* when egg masses were inoculated 15 days prior to transplanting and fungus at transplanting time in absence or presence of neem cake. This treatment when given in neem cake amended pots resulted into better plant growth than when given in the pots without neem cake.

Saikia *et al.* (2007) studied the efficacy of organic amendments *viz.*, neem cake, vermicompost, neem seed kernel, saw dust and carbofuran alone and in combinations against *M. incognita* in brinjal under field conditions. They reported that all the treatments significantly increased the plant growth parameters and yield of brinjal and decreased the nematode populations both in soil and roots. Among all the treatments, neem cake + carbofuran was found most effective in increasing the growth parameters and yield and reducing the nematode population.

In the modern age of intensive cropping, the problem of fungal diseases has become one of the important limiting factors for the crop production and therefore, the

use of chemicals for the management of plant diseases has become not only important but forms an essential component of various inputs for increasing the crop productivity. The first important landmark in the management of plant diseases caused by fungi with fungicides was the discovery of Bordeaux mixture by Millardet in 1885 (Agrios, 2005). Many hundreds of chemicals have been advanced to date for crop protection as fumigants, soil treatments, sprays, dusts, paints, pastes and systemics. Among them carbendazim (bavistin) and thiophanate methyl (tops-in-M) which belongs to benzimidazole group of fungicides, have the quality to control a large number of pathogens on many plant species at relatively low doses. These are used as foliar fungicides, post-harvest applications, seed and soil treatments. These have a common mode of action and inhibit fungal mitosis by interfering with spindle formation at the level of tubulin biosynthesis (Daniels *et al* , 1994) while topsin-M is actually responsible for toxic activity in fungi. These fungicides are effective against most of the pathogens belonging to Ascomycetes and some of the Basidiomycetes and Deuteromycetes such as *Botrytis*, *Cercospora*, *Colletotrichum*, *Gleosporium*, *Fusarium*, *Erysiphe*, *Phoma*, *Phomopsis*, *Pellicularia*, *Penicillium*, *Phyllosticta*, *Rhizoctonia*, *Sclerotinia*, *Uromyces*, *Venturia* etc. in different crops (Thind and Chahal, 2002).

El-razik *et al* (1990) tested ten fungicides to control the *F. oxysporum*, *F. moniliforme* and *R. solani* infecting onion under *in vitro* and green house conditions. They reported that all the test fungicides reduced fungal growth. However, treatment with bavistin and benomyl was found the most effective in reducing the disease development. Seed treatment with all the fungicides inhibited the growth of the three fungi under *in vitro* and protected seedlings from infection in green house trials.

Kapoor and Kumar (1991) studied the relative efficacy of systemic (benomyl, bavistin and topsin-M) and non-systemic (captan, captan and thiram) fungicides against 2 isolates each of *F. oxysporum* and *F. solani* from infected tomatoes by using the poison food technique. They reported that bavistin and benomyl were most effective treatments in controlling the disease. *F. solani* isolate KHF-41 was most sensitive and *F. oxysporum* isolate DF0-13 was least sensitive to these fungicides. *F. solani* isolates required 3-5 times higher doses of non-systemic fungicides than *F. oxysporum* isolates.

The decreasing order of overall efficacy of the fungicides was found in bavistin followed by benomyl, captafol, thiram, topsin-M and captan, respectively.

Etebarian (1992) tested the effect of 13 fungicides against the tomato wilt pathogen, *F. oxysporum* f. sp. *lycopersici* at the concentration of 10 and 100 ppm on PDA. Iprodione + bavistin, benomyl and bavistin totally inhibited the fungal growth after 10 days at concentration of 10 and 100 ppm. A field trial using soil drenching with iprodione + bavistin, bavistin and benomyl showed that benomyl was most effective in controlling the disease when applied @ 10 ppm followed by iprodione + bavistin and bavistin, respectively.

Ho *et al.* (1992) reported that benomyl as a seed or soil treatment and soil drenching with topsin-M gave the best control of *R. solani* on *Brassica chinensis* var. *parachinensis* in green house trials.

Chakrabarty (1993) determined the effect of 9 fungicides and 7 antibiotics against curd-rot disease complex associated pathogens viz., *Al. brassicae*, *B. cinerea*, *F. equiseti*, *P. tropica*, *P. aphanidermatum*, *R. solani* and *S. sclerotiorum* and a bacterium, *Erwinia carotovora* on cauliflower. He reported that the captafol, mancozeb, chloramphenicol, streptomycin and boric acid were found effective. The best control of the disease complex was achieved when the curds were pasted with a slurry of chloramphenicol + captafol (1:25) and subsequently sprayed with an aqueous suspension of this mixture @ 0.01 + 0.25%.

Morshed (1995) studied the effect of 6 fungicides on seedborne pathogens, viz., *C. lindemuthianum*, *Fusarium* spp., *Alternaria* spp., *B. cinerea*, *Ph. phaseoli* and *R. solani* and nodule formation of french bean under field conditions. He reported that bavistin, thiram and topsin-M were effective against all the test fungi and were compatible with *Rhizobium phaseoli*.

Al-Kassim (1996) determined the comparative efficacy of bavistin, benomyl, copper oxychloride + zinc, dithane M-45 and ridomil MZ-58 against 15 species of fungi belonging to the genera *Alternaria*, *Botrytis*, *Cladosporium*, *Curvuleria*, *Colletotrichum*, *Fusarium* and *Penicillium* isolated from locally cultivated seeds of okra, capsicum, radish and soybean. They reported that all the test chemicals significantly reduced the number of fungal species when seeds were treated with 0.2% concentration of each fungicide before

placing them on agar plates. Benomyl was found to be the most efficient seed treatment followed by copper oxychloride + zinc and dithane M-45, respectively in reducing the growth of the test fungi.

Javed *et al.* (1997) tested the efficacy of topsin-M, bavistin, benomyl and vitavax (each @ 3 g/kg) for their fungitoxicity against *F. solani* affecting onion seeds. They reported that bavistin gave the most effective control of the fungus. Seed germination was increased by vitavax and bavistin but that was statistically nonsignificant.

Gupta *et al.* (2000) studied the effect of various fungicides as seed treatment against root rot of french bean caused by *R. solani*. They reported that seed treatment with dithane M-45, mavistin, bavistin and alert was effective in controlling the disease 13.8, 15.7, 16.6 and 18.2%, respectively as compared to untreated control (28.3%).

Netam *et al.* (2002) reported that out of eight fungicides tested under *in vitro* against *F. oxysporum* f. sp. *solani* by using poison food technique in three concentrations viz., 550, 1000 and 1500 ppm, mycelial growth and sporulation were completely inhibited by bavistin at all the three concentrations. Lowest pre and post emergence mortality was also observed in bavistin (0 and 13%) over control.

De and Dwivedi (2003) determined the effect of metalaxyl, captan, bavistin, copper sulphate, topsin-M, thiram, carbofuran, carbosulfan and phorate against *F. oxysporum* f. sp. *lentis* under *in vitro* conditions. They reported that bavistin and captan @ 10 µg/ml was most effective in inhibiting (100%) the growth of the fungus.

Saxena *et al.* (2004) studied the effect of non-systemic fungicides viz., captan and thiram and systemic fungicides viz., vitavex and bavistin @ 10, 50 and 100 µg/ml concentrations on the radial growth of root rot pathogen of soybean, *R. solani*. They reported that all the fungicides invariably inhibited radial growth of *R. solani* irrespective of their concentrations. Among the non-systemic fungicides captan was found most effective fungicide by inhibiting the complete radial growth of the pathogen at 50-µg/ml concentration followed by thiram at 100 µg/ml. However, systemic fungicides vitavex and bavistin inhibited the complete radial growth of the pathogen at 10 µg/ml only.

Bharath *et al.* (2005) studied the effect of topsin-M, bavistin, dithane M-45, captan and blitox @ 0.2% concentration each as seed dressing against seed borne pathogens viz., *Ac. cucurbitacearum*, *Ac. cucumerina*, *Didymella bryoniae*, *F.*

oxysporum, *F. solani*, *F. equiseti*, *F. verticilloides* and *Myrothecium verrucaria* on watermelon under *in vitro*, pot and field conditions. They reported that bavistin was found highly effective against *Fusarium* spp. and dithane M-45 against *D. bryoniae*. However, topsin-M was found effective against all the fungal pathogens. Seed germination and vigour index was increased significantly by the treatment with bavistin and topsin-M under pot conditions. Whereas, topsin-M and dithane M-45 was found best in over all performance under field conditions.

Rathore (2006) studied the effect of bavistin, topsin-M, captan, captafol and thiram as seed treatment against root-rot and leaf blight of green gram caused by *M. phaseolina* under sick field conditions. Seed treatment with bavistin (@ 2 g/kg seed) was highly effective in reducing the disease incidence (14%) and increasing the grain (1.32 q/ha) and fodder (10.14 q/ha) yields. However, topsin-M (@ 2 g/kg seed) was at par with that of bavistin.

Sarkar and Saxena (2007) studied the management of seed mycoflora viz., *As. niger*, *As. flavus*, *F. udum*, *Alternaria* spp., *Cur. Lunata*, *R. solani*, *M. phaseolina* and *Colletotrichum* sp. of sunhamp by using bavistin, thiram, metalaxyl, dividend and mancozeb as seed treatment (each @ 2 g/kg seed) under *in vitro* conditions. They reported that thiram and bavistin reduced the seed mycoflora, retained the viability and germination and improved the seedling vigour significantly.

Devi *et al.* (2008) studied the efficacy of fungicides viz., bavistin, mass M-45, ridomil MZ-72 and vitavax on mycelial growth of *R. solani* and *F. oxysporum* under *in vitro* conditions. They reported that bavistin was to be the most effective in reducing the mycelial growth of *R. solani* (100%) at 100 ppm and 200 ppm followed by ridomil MZ-72 (62.6 ± 1.26), mass M-45 (67.9 ± 0.49), and vitavax (100%) at 200 ppm, respectively. While in case of *F. oxysporum*, bavistin and vitavax were highly fungitoxic and 100% inhibited the mycelium at 100 ppm and 200 ppm concentrations.

Traditionally chemicals are used to manage the plant diseases, however, their excessive use has posed a threat to environment, animal and human health. Also the chemicals required, often are not within the reach of farmers in most of the developing countries (Agbenin *et al.*, 2004). Decomposition of organic matter in soil is known to influence microbial activity (Khanna and Singh, 1974). In past, considerable efforts were

made on alternative methods of disease management including organic amendments in the form of composts, saw dust, oil cakes, dry crop residues, seed powder, extracts of different plant parts, etc. have been used against soil borne fungi particularly *Fusarium* spp. and *Rhizoctonia* spp. (Jarvis and Thorpe, 1981; Haseeb, 1983, 2003, 2004; Phae *et al.*, 1990; Narwal *et al.*, 1997; Singh and Singh, 1997).

Chakraborti and Sen (1991) studied the suppression of *Fusarium* wilt of muskmelon caused by *F. solani* by the amendments of mustard (2%), groundnut (1%), neem (2%) cakes and saw dust (1%) under *in vitro* conditions. Mustard cake and sawdust also decreased spore germination of the pathogen. Soil amendment with neem and mustard cakes and sawdust reduced wilt 80, 65 and 45 percent respectively. Whereas, highest reduction in the population of soil fungi and bacteria was found in mustard cake amended soil followed by saw dust, neem and groundnut cakes, respectively.

Lukade (1992) studied the effect of farmyard manure, press-mud cake, subabul, wheat straw, paddy straw, cakes of groundnut, neem and cotton against *M. phaseolina* causing root rot of safflower. He reported that all the treatments were significantly effective in reducing seedling mortality except groundnut and cotton seed cakes. Among all the treatments, wheat and paddy straw were found to be highly effective in reducing the seedlings mortality.

Kazmi *et al.* (1995) determined the effect of neem oil on the growth of *M. phaseolina* under *in vitro* conditions. They found that neem oil was significantly effective against *M. phaseolina* and efficacy of neem oil was increased with increase in concentration.

Raj and Kapoor (1996) studied the effect of groundnut, mustard, sesamum and cotton seed cakes @ 0.25, 0.5, 1.0 and 2.0% each (w/w) as soil treatment against *F. oxysporum* f. sp. *lycopersici* on tomato cv. Pusa Ruby under pot conditions. They reported that groundnut and mustard cakes at 2.0% concentration were found most effective in reducing the disease incidence. However, maximum reduction in disease index was found in groundnut cake (77.1%) treated plants followed by mustard, sesamum and cotton seed cakes, respectively. Groundnut cake also improved the plant growth significantly.

Shivpuri *et al* (1997) determined the fungitoxic properties of ethanol extracts of 10 plant species viz *Allium cepa*, *A sativum*, *Azadirachta indica*, *Calotropis procera*, *Datura stramonium*, *Ocimum sanctum*, *Polyalthia longifolia*, *Tagetes erecta*, *Vinca rosea* and *Withania somnifera* against 5 pathogenic fungi viz, *A brassicola*, *C capsici*, *F oxysporum*, *R solani* and *S sclerotiorum* under *in vitro* conditions at two concentrations (500 and 1000 µg/ml). They reported that higher dose of plant extracts of *A indica*, *D stramonium*, *O sanctum*, *P longifolia*, *V rosea*, and *W sominifera* was relatively more effective in inhibiting the growth of all the test fungi

Ma *et al* (1999) determined the effect of compost extracts made from livestock manures of pig, horse and cow against wilt of cucumber caused by *F oxysporum* f. sp. *cucumerinum*. They reported 58.9-92.5%, 18.6-72.1%, and 38.5-72.8% inhibition of the growth of the fungus by manure of pig, horse and cow, respectively.

Basak and Lee (2000) studied the efficacy of cow urine and cow dung against Fusarium wilt of cucumber caused by *F oxysporum* f. sp. *cucumerinum* under *in vitro* conditions. They reported that germination of conidia and the percentage inhibition of mycelial growth were effectively decreased and varied greatly with respect to different hours and days of incubation and kinds of bio matters. The percent inhibition of mycelial growth was found more in cow dung than cow urine treatment.

Mathur and Gurjar (2002) studied the effect of extracts of 23 plants (@ 100%, 1:1 v/w) and 5 oil cakes (@ 1 g/ml) against chilli stem rot causing pathogen, *R. solani* under *in vitro* conditions. Maximum inhibition of mycelial growth of *R solani* was reported by the treatment of plant extracts of *Plantago ovata*, *A sativum*, *Trigonella foenum-graecum* and oilcakes of *Brassica juncea*. Whereas, *Gossypium*, *Azadirachta* and *Sesamum* cakes significantly inhibited the mycelial growth of *R solani*.

Cheuk *et al* (2003) determined the comparative efficacy of different batches of composts including tomato leaves, cull fruit, bark, sawdust and recycled compost coarse material against root rot fungus, *F oxysporum* f. sp. *radicis-lycopersici* on tomato under green house conditions. They reported that all the composts significantly reduced the disease and improved the yield.

Haseeb *et al* (2005 a) studied the effect of neem seed powder @ 100 kg/ha, chopped neem leaves and murraya leaves @ 300 kg/ha each, farm yard manure and mint

manure @ 1500 kg/ha each for the management of *F. oxysporum* on chilli under *in vitro* and pot conditions. They reported that all the treatment significantly inhibited the growth of *F. oxysporum* under *in vitro* conditions. Neem seed powder was found highly inhibitory to *F. oxysporum* followed by neem leaves, murraya leaves, mint manure and farmyard manure, respectively. Similar trend of reduction in percent root infection was also found under pot conditions.

Haseeb and Kumar (2006) determined the comparative efficacy of neem seed powder @ 100 kg/ha, chopped neem leaves and murraya leaves @ 300 kg/ha, farm yard manure and mint manure @ 1500 kg/ha for the management of *F. solani* on brinjal cv. PK 123 under *in vitro* and pot conditions. They reported that all the treatment significantly inhibited the growth of *F. solani* under *in vitro* conditions. Neem seed powder was found highly inhibitory to *F. solani* followed by neem leaves, murraya leaves, mint manure and farmyard manure, respectively. Similarly, under pot conditions, all the treatment significantly improved the plant growth and reduced the percent root infection as compared to untreated uninoculated control.

Maurya *et al.* (2008) studied the efficacy of organic amendments *viz.*, caster cake (2%), linseed cake (1%), neem cake (2%) and saw dust (1%) against *F. oxysporum* f. sp. *lycopersici* on tomato under *in vitro* and field conditions. They reported that all the organic amendments inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* under *in vitro* condition. Highest reduction in wilt incidence was observed in neem cake treated plants. However, linseed cake was found least effective in managing wilt of tomato.

Besides, the use of organic amendments, the use of biocontrol agents has been proved to be most effective means of management of the disease of fungal origin. Among the various biocontrol agents, the antagonistic fungi hold a great promise to manage various fungal diseases of plants (Cook and Baker, 1983).

Among various antagonistic fungi, few species of the genus *Trichoderma* have been used successfully against soil borne fungi especially *Fusarium* and *Rhizoctonia* infesting various vegetable crops (Elad *et al.*, 1980 a, b, 1983; Chet, 1987, 1990). Beside, inhibiting the growth of the fungi, *Trichoderma* spp. also promote plant growth (Inbar *et al.*, 1994) and have the ability to colonize root surfaces and the cortex (Kleifeld and Chet,

1992; Yedidia *et al.*, 1999). Various mechanisms such as antibiosis, competition, mycoparasitism and enzymatic hydrolysis have been suggested for the biocontrol activity *Trichoderma* spp. against phytopathogenic fungi (Sivan and Chet, 1992; Elad, 1995).

Intensive research and sustained effort over long period of time made clear that different isolates of *Trichoderma* from different soil samples have differential antagonistic capability against different pathogens (Maity and Sen, 1985). This emphasises the need for specific isolates of antagonist having broad-spectrum activity against large number of soil borne pathogens.

Calvet *et al.* (1990) studied the antagonistic effect of two isolates of *T. aureoviride* and 2 isolates of *T. harzianum* against *F. oxysporum* and *Verticillium dahliae* under *in vitro* test. They reported that both isolates of *T. harzianum* significantly inhibited the radial growth of both the test fungi.

Hartman and Fletcher (1991) determined the effect of nonpathogenic *F. oxysporum* isolates and *T. harzianum* against Fusarium crown and root rot disease of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici*. They revealed that nonpathogenic *F. oxysporum* isolates and *T. harzianum* reduced the disease severity significantly.

Monga (1993) studied the effect of nine *Trichoderma* spp. against cotton root rot pathogens, *R. solani* and *M. phaseolina* under *in vitro* conditions. They reported that one isolate of *T. harzianum* was effective against *R. solani* whereas, an isolate of *G. virens* showed antagonistic effect against *M. phaseolina*.

Mishra and Narain (1994) studied the antagonistic effect of four isolate of *G. virens* and one isolate of *Streptoverticillium* against *As. flavus*, *C. gloeosporioides*, *F. solani* and *R. solani* under *in vitro* conditions. Spore germination and radial growth of all the test fungi were inhibited by the cell free culture filtrates of all the bioagents.

Vonzenilkova *et al.* (1995) determined the efficacy of *T. harzianum* T3 and commercial preparation Th-102 and supresivit @ 100 ml as suspension of 10^5 cfu/ml against *Fusarium* spp. on cucumber. They reported that the application of both the isolates of *T. harzianum* reduced the disease and increased the yield of cucumber.

Padmodaya and Reddy (1996) determined the biocontrol potential of 10 isolates of *Trichoderma* spp. against tomato wilt causing pathogen, *F. oxysporum* f. sp.

lycopersici under *in vitro* conditions. They found that *T. viride* (H) was highly inhibitory to *F. oxysporum* f. sp. *lycopersici* in dual culture followed by *T. harzianum* (A.P.).

Bourbos *et al.* (1997) studied the effect of *T. harzianum* and *T. koningii* against *F. oxysporum* f. sp. *radicis-lycopersici* on tomato cv. Early pack no. 7 under green house conditions. They reported that *T. harzianum* and *T. koningii* @ 0.15 g/m² effectively controlled the disease and increased the yield significantly.

Bidari and Gundappagopal (1998) studied the effect of *T. viride* as seed treatment against *F. solani* on pigeonpea under field conditions and reported 27.62% reduction in wilt as compared to control.

Rajappan and Ramraj (1999) determined the efficacy of *T. viride*, *T. harzianum*, *T. hamatum*, *G. virens*, *P. fluorescens* and *B. subtilis* against the cauliflower wilt pathogen, *F. moniliformae* under *in vitro* conditions. They found that *T. harzianum* produced the maximum inhibition zone of 15 mm compared to the minimum of 7 mm by *T. hamatum* and there was no significant difference between the inhibition zones produced by *P. fluorescens* and *B. subtilis*. Whereas, soil application of talc-based formulation of *T. harzianum*, *P. fluorescens*, *G. virens* and *T. viride* effectively controlled the wilt of cauliflower under field conditions.

Kazempour *et al.* (2000) determined the antagonistic effect of *T. harzianum* (T1 and T2 from bean and tomato fields, respectively) and *T. viride* (T3 and T4, from a bean field) against Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* on tomato. They reported that the most effective antagonist was *T. harzianum* isolate T2 and the least effective was *T. viride* isolate T4. The antagonists had a positive effect on plant height and weight in diseased plants, but had only a slightly positive effect in uninfected plants.

Rahman *et al.* (2001) tested the antagonism of five isolates of *T. harzianum* against *R. solani* and *F. solani* causing seedling diseases of tomato. Significant variation was found among the isolates (TMG-1, TMG-2, TMG-4, W-120, and TK) in reducing radial growth of pathogens on dual culture plate technique. Seedlings mortality of tomato caused by *R. solani* and *F. solani* was reduced appreciably by all the test isolates. Fresh and dry weight of tomato seedlings were improved by the application of *T. harzianum* in soils. Performance of isolate TMG-2 was better in controlling the disease and also in promoting the growth of tomato seedlings.

Wagner and Kopacki (2002) tested ten isolates of *T. harzianum* for their abilities to inhibit the fungal pathogens viz, *F. oxysporum*, *F. solani* and *R. solani* infesting tomato. They reported that all the isolates inhibited the growth of the test pathogens under *in vitro* test. However various isolates of *T. harzianum* differed in their antagonistic effect against all the test pathogens in pot experiments. The best results were obtained by the use of *T. harzianum* isolate T-7, but the isolates T-21 and T-52 were also effective against the above pathogens. Three isolates showed weak antagonistic activities against *Fusarium* spp. and four to *R. solani*.

Bandyopadhyay *et al* (2003) determined the potential of various strains of *Trichoderma* species against major root pathogens viz, *Sclerotium* sp., *Fusarium* sp., *R. solani* and *M. phaseolina* under *in vitro* test. They found that all the strains of *Trichoderma* checked the growth of *Sclerotium* sp. but strain H-14 showed more sporulation than all other strains and checked 66.6% growth of *Sclerotium* sp. as compared to control. This strain also checked the growth of *Fusarium* sp. by 71.1%, *R. solani* by 73.3% and *M. phaseolina* by 51.1% respectively.

Verma and Dohroo (2004) studied the comparative efficacy of *T. viride* and *T. harzianum* against Fusarium wilt of pea caused by *F. oxysporum* f. sp. *pisi* under *in vitro* and field conditions. They reported that maximum growth inhibition of the pathogen was found with *T. viride* and *T. harzianum* under *in vitro* test and maximum disease suppression under field conditions. *T. viride* and *T. harzianum* also increased the seed germination and decrease the disease incidence as compared to untreated control.

Bharath *et al* (2005) studied the efficacy of *T. harzianum*, *T. virens* and *P. fluorescens* against seed borne fungal pathogens viz, *Ac. cucurbitacearum*, *Ac. cucumerina*, *Didymella bryoniae*, *F. oxysporum*, *F. solani*, *F. equiseti*, *F. verticilloides* and *My. verrucaria* on watermelon under *in vitro*, pot and field conditions. They reported that seed treatment with *T. harzianum* and *T. viride* improved the seed germination, seedling vigour and reduced the incidence of seed borne fungal pathogens. *T. harzianum* was found highly effective against *Fusarium* spp. However, *P. fluorescens* was found highly effective against *D. bryoniae*. All the biocontrol agents increased the yield significantly under field conditions.

John *et al.* (2006) studied the bioefficacy of *T. viride* and *As. niger* against *Fusarium* sp. and *Verticillium* sp. alone under *in vitro* and field conditions. They reported that both the biocontrol agents reduced the growth of both the pathogens under *in vitro* conditions by 81 – 90% and also reduced the percentage of wilt under field conditions.

Gandhi and Kumar (2006) studied the antagonistic effect of fungal biocontrol agents viz., *T. harzianum*, *T. viride* and *G. virens* against *R. solani* on potato. They reported that all the biocontrol agents inhibited the growth of *R. solani* significantly. Among all the treatments, *T. viride* (60%) was found highly effective in inhibiting the growth of *R. solani*.

Mathur *et al.* (2007) studied the effect of biocontrol agents viz., *T. viride* and *T. harzianum* as seed treatment and root dip treatment against *F. solani* on onion under field conditions. They reported that both the biocontrol agents reduced the disease incidence and increased the bulb yield. *T. harzianum* was found more effective in improving the yield.

Fluorescent pseudomonads, mainly *P. fluorescens* and *P. putida* are among the most abundant bacteria in the rhizosphere. These bacteria have attracted considerable attention since the end of the 1970s (Kloepper *et al.*, 1980), as they are able to promote the growth of cultivated plants (Schippers, 1992) and inhibit the growth or activity of plant pathogenic fungi (Weller, 1988; Barbosa *et al.*, 1995; Gomes *et al.*, 1996; Wei *et al.*, 1996). Considerable work has been carried out on the antagonistic activities of *P. fluorescens* against various phytopathogenic soil borne fungi on different crops (Weller, 1988; Gutterson, 1990; Upadhyay *et al.*, 2000; Haseeb, 2003, 2004; Zaidi *et al.*, 2004).

Frommell and Pazos (1993) studied the biocontrol potential of different bacteria isolated from the rhizosphere and phyllosphere of green house and field grown tomato plants tested against 11 fungal pathogens under *in vitro* conditions. They reported that among all the isolates tested, 28% of the rhizosphere and 18.4% of the phyllosphere isolates inhibited the growth of at least 1 pathogen. *P. fluorescens* and *P. putida* were most frequently isolated from the exorhizosphere and *Erwinia* sp. and *Clavibacter* sp. predominated in the endorhizosphere. *P. fluorescens*, *E. herbicola* and *Xanthomonas* sp. were more frequently found in the phyllosphere. A total of 80 bacterial strains inhibited mycelial growth and/or spore germination of *Fusarium* spp. or *Pythium* sp. The majority

of the bacteria that reduced disease incidence caused by *Fusarium* spp. and *Pythium* sp. also reduced mycelial growth (> 48%) and inhibited spore germination (68%).

Selvarajan and Jeyarajan (1996) studied the antagonistic potential of *P. fluorescens*, *B. subtilis*, *Laetisaria arvalis* and *Trichoderma* spp. against chickpea root rot pathogens, *F. solani* and *M. phaseolina* under *in vitro* conditions. They found that all the antagonists inhibited the radial growth of both the pathogens significantly and reduced the sporulation of *F. solani* and sclerotial size, germination and number of germ tube of *M. phaseolina*.

Padmodaya and Reddy (1998) investigated the efficacy of two bacterial antagonists *Pseudomonas* sp. and *B. subtilis* and 10 isolates of *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici* causing damping-off and wilt in tomato. They found that all the antagonists significantly reduced the damping-off and wilt when used as seed dressing or seed inoculants or seedling root inoculants. *T. viride* (H), *Pseudomonas* sp. and *Trichoderma* sp. (G) recorded at par healthy seedlings stand. However, in root dip treatment, *T. viride* (H) and *Pseudomonas* sp. recorded the least wilt disease index and in soil application, *T. viride* (H), *Trichoderma* sp. (G) and *T. viride* (M) isolates resulted in least disease index, which was superior than other antagonists.

Ram *et al* (1999) found that integration of *T. harzianum* with *P. fluorescens* resulted in suppression of rhizome rot of ginger caused by *P. myriotylum* and *F. solani* and increased germination, plant stand and yield.

Varshney and Chaube (1999) determined the efficacy of nine isolates of *P. fluorescens* isolated from rhizosphere of tomato against *R. solani*, *F. oxysporum* f. sp. *lycopersici*, *S. rolfii* and *Al. brassicae* under *in vitro* conditions. They reported that all the isolates significantly inhibited the growth of all the test pathogens. However, antagonistic potential of all the nine isolates differed significantly from each other.

Khan and Khan (2001) investigated the effects of soil application of *B. subtilis*, *P. fluorescens*, *Pen. digitatum*, *As. awamori* and *As. niger* on the plant growth, biomass production, yield and the rhizospheric population of *F. oxysporum* f. sp. *lycopersici* on tomato cv. Pusa Ruby in a field trial. They reported that plant growth and yield variables of uninoculated plants were increased after application of all the tested biocontrol agents. Application of *As. awamori* and *As. niger* resulted in 80% and 58% increase in the yield,

respectively. The yield (weight of fruits/plant) of plants inoculated with *F. oxysporum* f. sp. *lycopersici* was significantly increased by all the biocontrol agents especially with *As. niger* (53%), *As. awamori* (42%), *Pen. digitatum* (38%) and *B. subtilis* (28%). Application of all the biocontrol agents also decreased the rhizosphere population of the wilt fungus by 23-49%.

Agarwal *et al.* (2002) determined the efficacy of *P. fluorescens*, *B. subtilis*, *T. viride* and *T. harzianum* (each @ 4 g/kg seed) alone and in combinations against *F. oxysporum* f. sp. *ciceri* on chickpea cv. JG-62 and Ujjain-21 under field conditions. They reported that all the antagonists failed to reduce wilting of highly susceptible plants of chickpea cv. JG-62. However, disease incidence reduced significantly in less susceptible cultivar Ujjain-21. The minimum disease incidence (6.5%) was reported with *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *B. subtilis* + *P. fluorescens* followed by *B. subtilis* (6.9%), respectively.

Pande and Chaube (2003) studied the effect of isolates of *P. fluorescens* on the mycelial growth and sclerotial viability of *R. solani* under *in vitro* conditions. They found that the antibiosis of 6 isolates of *P. fluorescens* resulted in reduction of mycelial growth of *R. solani* and the inhibition zone ranged from 1.3 to 22.5 mm in different isolates on King's 'B' medium. Similarly 3.3 to 12.0 mm inhibition zone was recorded on PDA. Sclerotial inactivation studies found that pre-treatment of bacterial isolates to sclerotia for different length of time interval affected sclerotial viability under *in vitro* conditions.

Shanmugamaiah *et al.* (2005) studied the biocontrol potential of a *Pseudomonas* sp. against *R. solani*, *M. phaseolina*, *A. alternata*, *Pyricularia oryzae*, *Bipolaris oryzae* and *Cur. lunata* under *in vitro* conditions. They reported that the cell free culture filtrate of *Pseudomonas* sp. significantly inhibited the conidial germination and mycelial growth of most of the phytopathogenic fungi.

Haseeb *et al.* (2006 a) studied the effect of biocontrol agents viz., *P. fluorescens*, *As. niger*, *T. harzianum*, *T. virens* and *P. lilacinus* (@ 50 kg/ha each containing 10⁸ cfu/g culture) against *F. oxysporum* on chilli under *in vitro* and pot conditions. They reported that all the treatments significantly inhibited the growth of *F. oxysporum* under *in vitro* conditions. Under pot conditions, highest plant growth and lowest root infection was

observed in *T. harzianum* treated plants followed by *T. virens*, *P. fluorescens*, *As. niger* and *P. lilacinus*.

It has now fully been realized that any of the single method among chemicals and non-chemical means, neither control the disease completely nor economically suitable to the farmers and large cultivation. In recent past efforts were made to integrate two or more than two methods to combat the disease successfully. Keeping in view the laminas of any single management option, there is a need to develop effective management options, which should be safe and economically, environmentally and technologically sound. Efforts were also been made by several workers for the development of integrated disease management strategies with emphasis on non chemical means for effective management of soil borne pathogens particularly *F. oxysporum* and *R. solani* attacking different vegetable crops (Elad *et al* , 1980 b; Lewis and Papavizas, 1980; Chet *et al* , 1982; Kumar and Dubey, 2001; Dubey, 2002; Haseeb, 2003).

Chattopadhyay and Sen (1996) conducted experiments to develop the integrated management of wilt of muskmelon caused by *F. oxysporum* under *in vitro*, pot and field conditions. They reported that *As. niger* strain-A27 and *T. viride* strain-T4 (both biocontrol strains) were antagonistic to *F. oxysporum*. Soil application of potash (KCl) @186.7 kg/ha, caused maximum percent disease reduction while seed treatment with *As. niger* strain-A27 @5 g/200 seeds caused maximum reduction of population of the fungus in rhizosphere soil. Both in pot and field experiments, seed treatment with *T. viride* strain-T4 @ 5 g/200 seeds and biocontrol @ 0.1% with soil application of KCl was found most effective in reducing the percent disease reduction (74.14%), pathogen population in soil and increased the plant growth followed by the combination of seed treatment of *T. viride* strain-T4 with soil application of KCl.

Sabet *et al* (2000) determined the effect of tolclofos-methyl, topsin-M and metalaxyl + copper oxychloride on the growth of pathogenic (*F. solani*, *R. solani* and *S. rolfsii*) and antagonistic fungi (*T. harzianum*, *Chaetomium globosum* and *Coniothyrium minitans*) under *in vitro* and pot conditions on tomato cultivars Castlerock and Ace. They have also evaluated the antagonistic effect of *T. harzianum*, *C. globosum*, *B. subtilis* and *P. fluorescens* on the growth of all the three test pathogens under *in vitro* conditions. They reported that all the treatment significantly inhibited the growth of the test

pathogens under *in vitro* conditions. A reduced dose of metalaxyl + copper oxychloride in combination with fungal and bacterial antagonists effectively reduced the disease. However, the efficacy of disease control by biocontrol agents differed with the cultivars.

Kumar and Dubey (2001) conducted an experiment for the management of collar rot of pea caused by *F. solani* f. sp. *pisi* through the integration of biocontrol agents viz., isolates of *T. viride*, *T. harzianum* and *G. virens* and fungicides viz., bavistin, benlate, captan, topsin-M, thiram and vitavax under *in vitro* and field conditions. They reported that Ranchi isolate of *T. harzianum* and *G. virens* were found superior over other isolates to inhibit the mycelial growth of the pathogen. *T. harzianum* showed the maximum growth around the treated seeds followed by *G. virens*, thiram + *G. virens*, carboxin + *G. virens*, captan + *G. virens* and captan + *T. harzianum*, respectively. Seed treatment with captan (1 g/kg seed) + *T. harzianum* (10^6 cfu/10g seed) gave good germination, least disease incidence along with highest green pod yield, which was statistically similar with *T. harzianum* alone, thiram + *T. harzianum*, carboxin + *G. virens* alone treatment.

Dubey (2002) studied the effect of thiram, vitavax, captan and *T. viride* alone and in combination as seed treatment against collar rot of french bean disease caused by *M. phaseolina* under field conditions. He reported that all the treatments significantly reduced the disease incidence, increased seed germination and pod yield over untreated control. Maximum seed germination, minimum disease incidence with highest pod yield was found in plots treated with vitavax + *T. viride* followed by thiram + *T. viride* and captan + *T. viride*, respectively in both the years of study. Seed treatment with *T. viride* alone was found superior to fungicides treatment alone. Of the fungicides, vitavax treated seeds showed superiority over thiram and captan respectively in all respects.

Khan and Mehnaz (2003) studied the effect of *T. harzianum* and fungicides (bavistin, topsin-M, thiram and captan) each @ 2 g/kg seed alone and in combinations as seed treatment against mix inoculum of *F. oxysporum* f. sp. *lentis* and *R. solani* each @ 5 g/kg soil on lentil under pot conditions. They reported that all the treatments alone and in combination significantly reduced the pre and post emergence mortality and maintained the better plant stand.

Rehman *et al.* (2003) conducted a field experiment during summer and kharif seasons of 1997-98 and 2000-01 to determine the effect of soil solarization, captan and

biocontrol agents viz., *T. harzianum*, *T. viride*, *P. fluorescens* and *A. chroococcum* for the management of damping-off of tomato, chilli and brinjal caused by *Pythium*, *Fusarium* and *Phytophthora* spp. They reported that lowest incidence of the disease in tomato (3.9%), brinjal (1.5%) and chilli (1.3%) was found due to soil solarization with white polythene sheet for 30 days during the peak of summer followed by seed treatment with captan. Among the antagonists, *T. viride* recorded the lowest incidence of damping-off (5.7% in tomato, 4.7% in brinjal and 4.7% in chilli) followed by seed treatment with *P. fluorescens*, *T. harzianum* and *A. chroococcum*, respectively, which was at par with each other.

Kapoor *et al.* (2006) studied the effect of biocontrol agents viz., *T. koningii* (RMA-8) and *T. harzianum* (SMA-4) and biopesticide trichogard (each @ 2.5 kg/65 kg farmyard manure/ha), neem based biopesticide wanis @ 0.1, *Lantana camera* @ 10 t/ha and spray with bavistin alone and in integration against root rot- wilt disease complex on pea. They reported that among the different integrated treatment combinations, the combined treatment with *L. camera* amendment @ 10 t/ha + biocontrol agent trichogard @ 2.5 kg/65 kg farmyard manure/ha + spray with bavistin at the flowering stage was most effective in managing the root-rot-wilt disease complex of pea.

Gade *et al.* (2007) studied the integrated effect of soil solarization with pesticides viz., thiram, benomyl, *T. harzianum* and *P. fluorescens* against *F. udum* on pigeon pea under field conditions. They reported that soil solarization alone and its combinations with thiram + benomyl (1:1) @ 3 g/kg soil reduced wilting to the extent of 22.8, 22.6% during first year and 16.3 and 15.7% during second year, respectively. Among bioagents *T. harzianum* @ 4 g/kg seed had significantly reduced the wilt incidence (52.7 and 52.1%) during first and second year, respectively.

In the soil ecosystem, numerous inter relationship between microbial communities, host plants and pathogens/nematodes take place. The complexity of these interrelationships, and to a greater extent our lack of knowledge of their structure, prevents development of a simple classification for functional microbial communities (Vilich and Sikora, 1998). Several strategies including chemical and non-chemical means have been developed to manage the disease complexes caused by soil borne plant

pathogens by several workers (Arjunan *et al.*, 1987; Weller, 1988; Hoitink and Boehm, 1999).

Walia *et al.* (1994) studied the effect of green manuring on *R. solani*, *M. phaseolina* and *M. javanica* disease complex on tomato cv. HS-101 under pot conditions. They reported that the plant growth was significantly increased in soil amended with subabool and neem leaves compared with the unamended control. *M. javanica* population, number of galls and egg masses was also reduced in amended soil. Soil amendments with neem leaves decreased the incidence of both *M. phaseolina* and *R. solani* in the treatment where nematodes were inoculated one week earlier than the fungus. Whereas, amendment with the leaves of subabool reduced fungal disease only in treatment where *R. solani* was inoculated alone.

Haque *et al.* (1995) studied the effect of pesticides viz., bavistin, PCNB, benomyl, topsin-M, captan and carbofuran and biocontrol agents viz., *V. chlamydosporium* and *P. lilacinus* alone and in combinations against *M. javanica*, *M. phaseolina*, *R. solani* and *F. solani* disease complex on okra. They reported that *V. chlamydosporium*, *P. lilacinus*, benomyl and topsin-M were more effective than carbofuran against *M. javanica* when applied alone. Whereas, combined use of benomyl with topsin-M was found more effective in controlling infection of *M. javanica*, *M. phaseolina* and *F. solani* disease complex than the use of carbofuran with fungicides. They also reported that all the fungicides reduced the efficacy of biocontrol agents in controlling root rot-root-knot infection on okra.

Mehta *et al.* (1995) studied the effect of neem, mustard and cotton oil cakes and oatmeal (@ 3% w/w) against *M. javanica*, *M. phaseolina* and *R. solani* disease complex of tomato. They reported that plant growth was improved significantly in all the amendments. Amendment with mustard cake resulted in maximum plant weight compared to rest of the amendments, irrespective of *M. phaseolina* or *R. solani* inoculation. Neem cake was proved least effective. Oil cakes were able to reduce the nematode population. The number of galls were found least in plants amended with mustard cake. Whereas, *R. solani* was decreased only in cotton cake and oatmeal amended soil.

Arya and Saxena (1998) studied the biocontrol potential of *Trichothecium roseum* @ 1 g/kg soil and *T. viride* @ 5 g/kg soil against *R. solani* and *M. incognita* complex on tomato cv. Pusa Ruby under pot conditions and reported that both the biocontrol agents reduced the harmful effects of *R. solani* and *M. incognita* and improved germination significantly.

Siddiqui *et al.* (1999) studied the effect of root dip treatment with *P. aeruginosa* and *Trichoderma* sp. alone and in combination for the control of root-knot-root-rot disease complex caused by *F. solani*, *R. solani* and *M. javanica*. They reported that root dip treatment with *P. aeruginosa* or without *T. harzianum*, *T. koningii* and *T. hamatum* decrease the infection of roots by the test pathogens and increased the plant growth significantly.

Khan and Akram (2000) studied the effect of soil application of *P. lilacinus*, *G. virens*, *P. fluorescens* PRS-9, *B. polymyxa* and aldicarb + thiram against disease complex caused by *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on the growth and yield of tomato under field conditions. They reported that the highest increase in plant growth and yield was found in plants treated with *P. fluorescens* followed by pesticides, *G. virens* and *P. lilacinus*, respectively.

Siddiqui (2000) tested the antagonistic activity of *P. aeruginosa* strain IE-6, towards *M. javanica*, *M. phaseolina*, *F. solani* and *R. solani* under *in vitro* and greenhouse conditions. He indicated that cell-free culture filtrate of the bacterium caused significant reduction in egg hatching of *M. javanica* and inhibited radial growth of fungi under *in vitro* tests. Cell-free culture filtrate also caused lyses in mycelium of *F. solani*. Under greenhouse conditions, soil drenches with the aqueous cell suspension or cell-free culture resulted in a considerable reduction in nematode population densities in soil and subsequent root-knot development. In addition to nematode control, rhizobacterium application also inhibited root infection caused by all the three fungi with significant enhancement of tomato seedling growth.

Siddiqui *et al.* (2001) determined the biocontrol potential of *P. aeruginosa* strain IE-6 against *M. javanica* (0, 250, 500 and 1000 eggs/plant) and *R. solani* (0, 1 and 3 ml culture suspension/kg soil) disease complex on tomato cv. SUN 6002 under greenhouse experiments. They reported that bacterium suppressed root infection caused by *R. solani*

and *M. javanica* on tomato in both sterilized and non-sterilized soils and increased the plant growth. Highest biocontrol effects was found at low population levels of the pathogens than at higher population densities. The severity of root-rot disease was more pronounced in sterilized soil as compared to the non-sterilized soil. Root colonization by *R. solani* and other 3 root-infecting fungi including *M. phaseolina*, *F. oxysporum* and *F. solani* was also lower in the presence of *P. aeruginosa* in non-sterilized soil.

Chand and Tripathi (2002) determined the effect of different agrochemicals on the population density of *M. incognita*, *R. solani*, *T. viride*, *T. harzianum*, *T. longitrichum* and *G. virens*. All the chemicals, except carbaryl and isoproturon significantly affected the population of *R. solani*. Application of topsin-M reduced colonies of *R. solani* from 7.66×10^4 to 1.33×10^4 . Similarly, 3.33×10^4 colonies of *T. viride* and 2.00×10^4 colonies of *T. harzianum* were recorded by the application of topsin-M. Topsin-M also reduced the number of juveniles of *M. incognita* from 623.66 to 127.33 J₂/200 g soil. Insecticides and herbicides showed less influence on population density of *R. solani* and antagonists, whereas insecticides showed significantly better response against *M. incognita*.

Haseeb *et al.* (2003 a) determined the efficacy of neem seed powder @ 50 kg/ha along with carbofuran and bavistin @ 2.0 kg a.i./ha for the management of *M. incognita* and *F. oxysporum* on okra cv. Arka Anamika under pot conditions and observed that neem seed powder performed almost equal to carbofuran and *T. harzianum* in increasing the plant fresh weight. However, neem seed powder was found inferior to carbofuran and bavistin in suppressing nematode reproduction and root colonization by fungus.

Haseeb *et al.* (2004) determined the comparative efficacy of *P. fluorescens*, *As. niger*, *T. harzianum*, *T. virens* and *P. lilacinus* @ 50 kg/ha, neem seed powder @ 50kg/ha, chopped neem leaves and murraya leaves @ 3.0 q/ha, farm yard manure and mint manure @ 30 t/ha, carbofuran and topsin-M @ 2.0 kg a.i./ha for the management of *M. incognita* and *F. oxysporum* on brinjal cv. Pusa Kranti. They observed that all the treatments were effective in increasing the plant fresh weight as compared to untreated inoculated plants.

Haseeb and Kumar (2005) conducted an experiment for the evaluation of biocontrol agents viz., *P. fluorescens*, *As. niger*, *T. harzianum*, *T. virens* and *P. lilacinus*

@ 50 kg/ha each containing 10^8 cfu/g culture; organic amendment materials viz., neem seed powder @ 100 kg/ha, fresh leaves of neem and murraya @ 300 kg/ha, farmyard manure and mint manure @ 1500 kg/ha, and pesticides viz., carbofuran and topsin-M @ 2.0 kg a.i./ha for the management of *M. incognita* (@ 4000 J₂/4 kg soil) and *F. solani* (@ 10 g/4 kg soil containing 10^6 cfu/g culture) disease complex on brinjal cv. Pusa Kranti under pot conditions. They reported that all the treatments significantly improved the plant growth and reduced the percent root infection and root-knot index as compared to untreated inoculated control except the treatment with farmyard manure. *T. harzianum* was found most effective in improving the plant growth followed by *T. virens*, *As. niger*, *P. fluorescens*, *P. lilacinus*, carbofuran, neem seed powder, topsin-M, neem leaves, murraya leaves and mint manure, respectively.

Haseeb *et al* (2006 b) studied the effect of biocontrol agents viz., *P. fluorescens*, *As. niger*, *T. harzianum*, *T. virens* and *P. lilacinus* (each @ 50 kg/ha containing 2×10^8 cfu/g culture); organic amendments viz., fresh chopped neem leaves and murraya leaves @ 3.0 q/ha each, farmyard manure and mint manure @ 15 q/ha each and neem seed powder @ 1 q/ha) and pesticides (carbofuran and topsin-M @ 2 kg a.i./ha each) against *M. incognita* (2000 J₂/4kg soil) - *F. solani* (7 g culture having 10^6 cfu/4 kg soil) disease complex of tomato cv. K-25 under pot conditions. They reported that all the treatments improved the plant growth and fruit yield significantly as compared to untreated inoculated plants. *T. harzianum* was found superior among all the treatments in improving the fruit weight and plant growth followed by *P. fluorescens*, carbofuran, *As. niger*, *T. virens*, neem seed powder, topsin-M, *P. lilacinus*, neem leaves, murraya leaves, mint manure and farmyard manure, respectively. Carbofuran was found highly effective against *M. incognita*, topsin-M against *F. solani* and *T. harzianum* was found effective against both the pathogens.

Haseeb *et al* (2007 a) studied the efficacy of pesticides (carbofuran and bavistin, each @ 1 mg a.i./kg soil), organic amendments (neem seed powder @ 50 mg a.i./kg soil and murraya leaves powder @ 150 mg a.i./kg soil) and biocontrol agents (*T. virens* and *P. fluorescens*, each @ 50 ml/kg soil containing 10^8 cfu/ml culture) against *M. incognita* (2500 J₂/kg soil) - *F. oxysporum* (2.5 ml/kg soil having 10^8 cfu/ml) disease complex of field pea cv. Rachna under pot conditions. They reported that all the treatments

significantly improved the growth of plants as compared to untreated inoculated plants. Carbofuran and bavistin was most effective in suppressing the root-knot development and fungal infection respectively, while neem seed powder and *T. virens* were found effective against both the pathogens.

Till recent past, use of chemicals was considered as the most effective means to overcome the soil borne pathogens. However, increasing use of chemical pesticides for management of soil borne plant pathogens including plant parasitic nematodes had caused pollution of soil, surface and ground water besides affecting the crop produce and ecosystem and also develop the resistance in pathogens. In this context, innovative approaches with limited use of chemicals are coming up as an alternative strategy for disease management, which is also ecology conscious and environment friendly. Efforts were made towards formulation of management systems by and large have been aimed against monopathogenic situations, although the occurrence of disease complexes involving plant parasitic nematodes is not uncommon in nature (Powell, 1979). It is now widely recognized that integrated approach is an essential and most desirable feature of managing diseases of complex nature, which includes appropriate blending of various management technologies to meet the needs of farmers (Andrews, 1983; Papavizas and Lewis, 1988; Muthamilan and Jeyarajan, 1996; Upadhyay and Dwivedi, 2000). Attempts have been made to develop integrated approach with emphasis on biocontrol agents and botanicals for effective management of *Fusarium*, *Rhizoctonia* and *Meloidogyne* disease complexes on vegetable crops (Stephan *et al.*, 1996; Singh and Goswami, 2001; Haseeb *et al.*, 2003 b, 2005 c, d).

Stephan *et al.* (1996) studied the effect of *P. lilacinus* and a mutant isolate of *Trichoderma* sp. on the management of root-knot-wilt disease complex of tomato caused by *M. javanica* and *F. oxysporum* f. sp. *lycopersici*. They reported that the highest decrease in disease and root gall index and increase in plant height and shoot dry weight was observed in plants treated with a combination of either *Trichoderma* sp.-oxamyl or *Trichoderma* sp.-fenamiphos followed by treatments with *P. lilacinus*-*Trichoderma* sp. or benomyl-*Trichoderma* sp. respectively. They have also observed that the nematicides used were inhibitory for the growth and activity of *Trichoderma* sp.

Singh and Goswami (2001) determined the efficacy of neem cake and/or carbofuran against *M. incognita* and *F. oxysporum* disease complex on cowpea cv. Pusa Komal. They reported that application of neem cake and carbofuran significantly increased the plant growth and reduced the nematode multiplication. Treatment with combination of neem cake and carbofuran in reduced doses (@ 0.5% w/w and 0.75 kg a.i./ha respectively) gave the best response in reducing the nematode multiplication with an increase in plant vigour.

Chaitali *et al.* (2003) determined the effect of neem and groundnut cakes (@ 5% w/w each) with *T. viride* (@ 2 g mycelial mat/500 g soil containing 5×10^6 cfu/g mycelium mat) for the management of disease complex caused by *M. phaseolina* and *M. incognita* on okra cv. Pusa Sawani. They reported that all the treatments reduced the nematode population and significantly improved the plant growth as compared with untreated control. The neem cake was found highly effective followed by *T. viride* and groundnut cake, respectively. However, the combined effect of *T. viride* with neem cake was found most effective than *T. viride* with groundnut cake.

Haseeb *et al.* (2003 b) conducted nursery trials in farmer's field at Hathras for the management of root-knot-wilt disease complex of chilli cv. Jwala during December and January 2001 and July and August 2002 by the application of carbofuran and topsin-M @ 2.0 (full dose) and 1.0 (half dose) kg a.i./ha, *As. niger* and *T. harzianum* at 50 (full dose) and 25 (half dose) kg/ha (10^8 cfu/g) alone and in different combinations. They reported that root-knot and fungal infection was completely checked by the treatment with carbofuran + *T. harzianum*/*As. niger* and carbofuran + topsin-M when applied as full doses. Other treatments were also effective at varying degree. Highest number of transplantable seedlings and total weight of seedlings were recorded in plots treated with carbofuran + *T. harzianum* (full dose) followed by carbofuran + *As. niger* (full dose), carbofuran + topsin-M (full dose), carbofuran + *T. harzianum* (half dose), *T. harzianum* + *As. niger* (full dose), carbofuran + *As. niger* (half dose), carbofuran + topsin-M (half dose), *T. harzianum* + *As. niger* (half dose), carbofuran (full dose), *T. harzianum* (full dose), topsin-M (full dose) and *As. niger* (full dose), respectively.

Haseeb *et al.* (2005 c) studied the comparative efficacy of combinations of full and half doses of carbofuran (33.0 kg/ha), topsin-M (2.0 kg/ha), neem seed powder

(100.0 kg/ha). suspension of *P. fluorescens* and *T. harzianum* having 10^8 cfu/ml (50.0 l/ha) applied as full dose before transplanting or in split doses before and 45 days after transplanting for the management of *M. incognita* and *F. oxysporum* disease complex on brinjal cv. BSS-332. They revealed that all the treatments were effective in increasing the fruit yield as compared to untreated plots. Highest fruit yield was recorded in plots treated with *T. harzianum* (50.0 l) + carbofuran (16.5 kg) followed by carbofuran (33.0 kg) + topsin-M (1.0 kg), topsin-M (2.0 kg) + carbofuran (16.5 kg), neem seed powder (100.0 kg) + carbofuran (16.5 kg), *T. harzianum* (50.0 l) + neem seed powder (100.0 kg), *P. fluorescens* (50.0 l) + carbofuran (16.5 kg) and *T. harzianum* (50.0 l) + *P. fluorescens* (50.0 l), respectively. Application of treatments in split doses was more effective than full doses applied before transplanting. Suppressive effect of various treatments on extent of infection in roots was significant till 90 days after transplanting.

Haseeb and Kumar (2008 a) studied the effect of two biocontrol agents viz, *T. virens* and *P. lilacinus* (each @ 10^9 cfu/m²) alone and two organic amendments viz, neem seed powder (@ 50 g/m²) alone and farmyard manure (@ 300 g/m²) alone and integrated treatments against *M. incognita*-*F. solani* disease complex on chilli cv. Patna under nursery and field conditions. They reported that highest percent emergence of seedlings (87) and fresh weight of total seedlings per bed (180.0 g) was found in *T. virens* + *P. lilacinus* + neem seed powder + farmyard manure treated plants followed by *T. virens* + *P. lilacinus* + neem seed powder, *T. virens* + neem seed powder + farmyard manure, *T. virens* + *P. lilacinus* + farmyard manure, *T. virens* + neem seed powder, *T. virens* + *P. lilacinus*, *P. lilacinus* + neem seed powder + farmyard manure, *T. virens* + farmyard manure, *P. lilacinus* + neem seed powder, *T. virens* alone, *P. lilacinus* + farmyard manure, neem seed powder alone, *P. lilacinus* alone and farmyard manure alone, respectively as compared to untreated beds. Lowest root-knot index (0.9) and root infection by *F. solani* (10.0%) was also found in *T. virens* + *P. lilacinus* + neem seed powder + farmyard manure treated plants. Similarly, in field experiment, highest yield (81.3 q/ha green chilli and 22.0 q/ha red chilli), lowest root-knot index (1.3) and percent root infection by *F. solani* (15.5) was also observed in *T. virens* + *P. lilacinus* + neem seed powder + farmyard manure treated plants.

CHAPTER – 3

MATERIALS AND METHODS

1. Survey for the occurrence, distribution and identification of nematode and fungi, causing disease complex in tomato, and for collection of naturally occurring fungal and bacterial antagonists from western districts of Uttar Pradesh

Comprehensive survey of various localities of Agra, Aligarh, Bulandshahar and Mathura districts located in the western part of Uttar Pradesh were carried out in the months of April 2004 and March 2005 by taking soil and root samples from the rhizosphere of apparently diseased plants of tomato (*Lycopersicon esculentum*) for identification of the prevailing species of plant parasitic nematodes and soil borne fungi and to determine the extent of infection. For biocontrol agents particularly species of *Trichoderma* and *Pseudomonas fluorescens*, the soil samples were collected from the rhizosphere of healthy plants found in diseased fields.

1.1. Symptomatology

The above and below ground disease symptoms produced by the nematodes and fungi were observed and recorded.

1.2. Collection of soil samples

Soil samples were collected with the help of auger (khurpi) from the rhizosphere of tomato plants. From each field 5-20 sub samples were taken randomly according to the area of the field. The sub samples were mixed thoroughly and approximately 500 g soil was placed into polythene bags and tagged with relevant information. The samples were brought to the laboratory and kept in refrigerator (4°C) until the plant parasitic nematodes, phytopathogenic fungi and biocontrol agents particularly species of *Trichoderma* and *P. fluorescens* were isolated separately.

1.3. Isolation and identification of plant parasitic nematodes and fungi from soil

1.3.1. Isolation of nematodes

Nematodes were isolated from soil using Cobb's sieving and decanting technique in conjunction with Baermann funnel (Southey, 1986). A 250 g soil sample was placed into a plastic bucket, and it was filled with ten liters of water. The suspension was stirred

gently and thoroughly to break the soil aggregates and release nematodes into water. The suspension was allowed to settle for 2 min for heavy soil particles to sink in to the bottom of the bucket. The suspension was decanted through sieves with the pore openings 710, 63, 45, 39 μm . The entire process was repeated twice for improved nematode recovery. Thereafter, the nematodes and remaining debris were collected in a beaker by gently rinsing from the sieve.

To obtain a clean suspension of the nematodes, the Baermann's funnel method was employed. A double folded fine tissue paper was placed on sieves with openings of 710 μm in Baermann funnel. The funnels were placed on support and water was added. The suspension containing nematodes was poured over the tissue paper. The nematodes being active made their way through the tissue paper into the water. After 48 h the nematodes were recovered by opening the clip of the rubber tube connected to the bottom of the Baermann funnel, thus allowing the nematodes in water to flow out in a beaker and final volume of the suspension was made up to 100 ml by adding tap water.

1.3.2. Identification and counting of plant parasitic nematodes

The identification of plant parasitic nematodes was done under the microscope with the help of the following literature:

- (i) Tylenchida: Parasites of Plants and Insects, 2000. Edited by M.R. Siddiqi, published by Commonwealth Agricultural Bureau's, U.K.
- (ii) Description of Plant Parasitic Nematodes, published by Commonwealth Institute of Helminthology, U.K.
- (iii) Pictorial Key to Genera of Plant Parasitic Nematodes, 1975. Edited by W.F. Mai and H.H. Lyon, Comstock publishing associates, Ithaca and London.
- (iv) Plant Nematology, 1982. Edited by J.F. Southey, A.D.A.S. Plant Pathology Laboratory, Harpenden, U.K.
- (v) Introduction to Research on Plant Nematology, 1971. A FAO Guide to the Study and Control of Plant Parasitic Nematodes. Edited by A. L. Taylor. FAO, Rome.
- (vi) Various national and international journals of repute.

For counting the nematodes, 1 ml nematode suspension was poured into a shallow multichambered counting dish and observed under the microscope (Doncaster, 1962). The counting of each sample was done twice to reduce the error. From the resulting

nematode count, nematode population in 100 ml suspension i.e. 250 g soil was calculated.

1.3.3. Isolation of pathogenic fungi

Fungi were isolated from rhizospheric soil on potato dextrose agar (PDA) medium by using serial dilution plate technique, in which 1 g soil from each sample was added to 9 ml sterilized distilled water in test tube. The soil suspended in the tube shaken gently but thoroughly to mix soil particles and get them uniformly dispersed. 1 ml of the suspension from the first dilution (10^{-1}) was aseptically transferred to another tube (10^{-2}) containing 9 ml sterilized distilled water. This procedure was repeated till the original sample was diluted up to 10^{-4} . For isolation of fungi, 1 ml soil suspensions from the dilutions (10^{-3} and 10^{-4}) was transferred in sterilized petriplates containing PDA and gently rotated to ensure uniform distribution of the soil suspension. The plates were then incubated in BOD incubator at $27 \pm 1^\circ\text{C}$ for 5 days. Plates were observed regularly for growth of fungal colonies.

1.3.4. Identification of fungi

The colonies were identified separately by preparing the slides of each fungus. After that, the slides were observed under the microscope and compared by the morphological and cultural characters described in standard references.

1.4. Collection of root samples

Root samples were also collected and tagged simultaneously from apparently diseased plants of tomato, in the same manner as described for soil samples in section 1.2. These samples were also kept in refrigerator until the root-knot index and percent root infection by fungi were graded, and isolation of fungi and nematodes was done from the root samples.

1.5. Grading of root-knot index

The root samples collected from different localities were washed thoroughly under gentle stream of running tap water to remove the adhering soil particles and afterwards root-knot index was graded separately from individual locality on a scale of 0-4 (Taylor and Sasser, 1978), where:

0	=	No galling	(0%)
1	=	Light galling	(1% - 25%)

- | | | | |
|---|---|------------------|--------------|
| 2 | = | Moderate galling | (26% - 50%) |
| 3 | = | Heavy galling | (51% - 75%) |
| 4 | = | Severe galling | (76% - 100%) |



1.6. Isolation and identification of root-knot nematodes and pathogenic fungi from roots

1.6.1. Isolation and counting of root-knot nematodes from roots

Isolation of nematodes from roots was done by mechanical maceration. Thoroughly washed 5 gram roots from each sample were cut separately into small pieces and macerated in an electric warring blender with sufficient amount of water. The suspension was cleared by sieving through a 710 μ m openings sieve and the final volume of the suspension was made up to 100 ml by adding tap water (Southey, 1986).

Counting of the nematodes was done as the method described in 1.3.2, 1 ml nematode suspension was poured into a shallow multichambered counting dish and observed under the microscope (Doncaster, 1962). The counting of each sample considering all the developmental stages of the root-knot nematode was done twice to reduce the error. Nematode population in 100 ml suspension i.e. 5 g roots was calculated from the resulting nematode counts.

1.6.2. Identification of root-knot nematode (*Meloidogyne* species)

Ten fully mature female specimens of the nematodes were excised from the galled tissues of the roots of tomato, collected during survey from each and every locality. Perineal pattern of mature females were cut and stained in hot acid fuschin (0.01%) and mounted in lactophenol (Taylor and Netscher, 1974). Specific identification of the nematodes was done in the laboratory by the close examination of perineal pattern under a microscope (Southey, 1986).

1.6.3. Isolation and identification of pathogenic fungi from roots

Apparently infected portion of roots of tomato plants collected during survey were surface sterilized in 0.1% sodium hypochlorite solution and placed in petriplates containing PDA (Riker and Riker, 1936). The plates were then incubated in a BOD incubator at $27 \pm 1^\circ\text{C}$ and observed for the growth of fungi. Hyphae coming out the root pieces were sub-cultured on the fresh PDA in petriplates. Sub cultures were mostly

identified as *Fusarium* and *Rhizoctonia* species. The culture of *Rhizoctonia* was purified by single hyphal tip method and that of *Fusarium* by single spore method (Riker and Riker, 1936). The species identification was done by comparing the morphological and cultural characters as described by Mordue (1974) for *Rhizoctonia*, and Booth (1971) for *Fusarium*.

1.7. Determination of percent root infection by fungi

The washed roots were cut into pieces of 1.0 cm length and treated with 10% KOH solution and kept at 90°C in a hot air oven for 1 h. The root pieces were then washed again with distilled water, acidified and stained with trypan blue (0.05% in lactophenol) as described by Phillips and Haymen (1970). Ten stained root pieces were taken separately from the samples of individual locality and mounted on a slide in lactophenol and observed under microscope. The portion of length of root pieces, which showed the presence of hyphae of fungi, was estimated. The percent root infection was calculated by measuring the infected portion in relation to total length of root pieces (Biermann and Lindermann, 1981).

1.8. Isolation and identification of naturally occurring fungal and bacterial biocontrol agents

1.8.1. *Trichoderma* species

Isolation of *Trichoderma* species, was done from samples randomly collected from rhizospheric soil of healthy tomato plants and grown on *Trichoderma* selective medium (Elad and Chet, 1983) modified by Saha and Pan (1997) by using serial dilution plate technique. Cultures were grown on PDA slants at $27 \pm 2^\circ\text{C}$ and maintained at 4°C for further study. The specific identification of species of *Trichoderma* was made by comparing the morphological and cultural characters described by Bissett (1991). Later on, cultures of *T. harzianum* and *T. virens* collected from different localities were considered as separate isolates and designated as TH-AG-2, TH-M-7, TH-AG-5, TH-MN-2, TH-UIP-2, TH-JP-2, TH-SJ, TH-AL, TH-K-9, TH-H-3, TH-BS-6, TH-SP-1, TV-K-3, TV-H, TV-M-1, TV-AG-3, TV-M-5 and TV-AL-1.

1.8.2. *P. fluorescens* isolates

Soil samples were collected from rhizospheric soil of healthy tomato plants randomly from different localities to find out the different isolates of *P. fluorescens* by

serial dilution plate technique using King's 'B' medium (King *et al.*, 1954). Pure cultures of each isolate were grown on King's 'B' medium slants at $30 \pm 1^\circ\text{C}$ and maintained at 4°C for further study. These cultures were identified on the basis of morphological and cultural characteristics as described by Holt *et al.* (1994). Later on, cultures of *P. fluorescens* collected from different localities were considered as separate isolates and designated as AG-1, AG-4, RGP-1, BW-1, BW-2, PS-4, PS-5, PS-9, UIP-1, UIP-2, NK-1, NK-2, H-2, K-1, K-3, M-3, and M-5.

2. Pathogenicity test of *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* alone on tomato cv. K-25 under pot conditions

2.1. Collection of the seeds

Seeds of tomato (*Lycopersicon esculentum*) cv. K-25 were obtained from local market of Aligarh.

2.2. Raising of tomato seedlings

Surface sterilized seeds of tomato cv. K-25 were sown in 25-cm-top diameter clay pots containing 5 kg steam sterilized sandy loam soil (70% sand, 22% silt, 8% clay, pH 7.5) and farmyard manure (4:1) mixture. Pots were kept in the green house and watered as and when considered necessary.

2.3. Maintenance and production of culture of *M. incognita*

After identification of *Meloidogyne* species (described in 1.6.2), egg masses of *M. incognita* were picked up and surface sterilized in 1: 500 aqueous solution of sodium hypochlorite for 5 min and a single egg mass was transferred to a small coarse sieve lined with double layer of tissue paper, earlier placed in a petriplate containing sufficient amount of water (den Ouden, 1958). The petriplates were incubated at room temperature ($27 \pm 5^\circ\text{C}$) to get the second stage juveniles. Seedlings of brinjal, grown in autoclaved soil were inoculated with progeny of the single egg mass from time to time in order to get regular supply of the inoculum for experiments.

2.4. Mass culture of *F. oxysporum* and *R. solani*

Mass cultures of both the pathogenic fungi were maintained on potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks. Flasks, each containing 100 ml PDB, were autoclaved at 1 kg/cm^2 pressure for 30 min. The flasks were then allowed to cool at room

temperature, afterwards each flask was inoculated separately under aseptic conditions with 1-cm-diameter PDA discs punched from the periphery of actively growing 7-days-old cultures of *F. oxysporum* and *R. solani* separately. Later, flasks were placed in a BOD incubator at $27 \pm 1^\circ\text{C}$, and the fungus was allowed to grow for 7 days. Later on, the spore suspension of *F. oxysporum* was prepared by macerating the known amount of mycelial mat in a warring blender with sterilized distilled water (1:2 w/v) for 30 seconds. Later on, cfu were counted with the help of haemocytometer under microscope to maintain the 10^6 cfu/ml for experimental purpose. For *R. solani*, similarly the known amount of mycelial mat was blended with sterilized distilled water (1:2 w/v) in warring blender for 30 seconds.

2.5. Pathogenicity test of *M. incognita*

2.5.1. Transplantation and inoculation

20-days-old seedlings were transplanted singly in to 25 cm-top-diameter clay pots each containing 5 kg steam sterilized soil and farmyard manure (4:1) mixture. Three days after transplantation, seedlings were inoculated separately with predetermined number (0, 2500, 5000, 10000, 15000, 20000 and 25000) of J_2 of *M. incognita* in aqueous suspension by making 3-5 cm deep, 4-5 holes in a radius of 1.5 cm and pouring into the holes using a sterilized pipette. The holes were then plugged gently with soil. There were five replicates for each treatment and the experiment was laid out in completely randomized block design.

2.5.2. Recording of data

2.5.2.1. Plant growth and Fruit yield

Picking of ripened fruits was done on 110 day after inoculation. The number of fruits and weight were taken separately from each treatment and replicate. There after plants were carefully uprooted from pots and the roots were washed in running tap water to remove the adhering soil particles. Excess water was removed with blotting paper. Plant growth was determined by measuring shoot height, fresh and dry weights of shoot and roots. For determining dry weight, the shoot and roots were dried in an oven at 60°C for 24 h. The percent reduction in plant growth over uninoculated control was also calculated.

2.5.2.2. Estimation of nematode population

After termination of the experiment, soil and root samples were taken separately from each and every replicate for the estimation of nematode population. Isolation and counting of nematodes from soil and roots was done as described earlier in section 1.3.1, 1.3.2 and 1.6.1, respectively. Root-knot index was graded on 0-4 scale as mentioned in section 1.5.

2.6. Pathogenicity test of *F. oxysporum*

2.6.1. Transplantation and inoculation

For determining the effect of different initial inoculum levels of *F. oxysporum*, seedlings of tomato were transplanted in the same manner as described in section 2.5.1. Three days after transplantation, roots of tomato were exposed by removing the soil and predetermined amount (0, 2.5×10^6 , 5×10^6 , 7.5×10^6 , 10^7 , 1.25×10^7 , 1.5×10^7 cfu/5 kg soil) of *F. oxysporum* culture grown on PDB was mixed in soil around the exposed roots, afterwards roots were covered gently with sterilized soil. There were five replicates of each treatment and the experiment was laid out in completely randomized block design (RBD).

2.6.2. Recording of data

Recording of data regarding the plant growth and fruit yield and percent root infection was done as described in section 2.5.2.1 and 1.7 respectively.

2.7. Pathogenicity test of *R. solani*

2.7.1. Transplantation and inoculation

For determining the effect of different initial inoculum levels of *R. solani*, seedlings of tomato were transplanted in the same manner as described in section 2.5.1. Three days after transplantation, roots of tomato were exposed by removing the soil and predetermined amount (0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 g mycelium/5 kg soil) of *R. solani* culture grown on PDB was mixed in soil around the exposed roots, afterwards roots were covered gently with sterilized soil. There were five replicates of each treatment and the experiment was laid out in RBD.

2.7.2. Recording of data

Recording of data regarding the plant growth and fruit yield and percent root infection was done as described in section 2.5.2.1 and 1.7 respectively.

3. Interactive effect of *M. incognita*, *F. oxysporum* and *R. solani*, alone and in combination on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

3.1. Transplantation and inoculation

Transplantation of seedlings was done in the manner as described in section 2.5.1. After 3 days of transplantation, predetermined amount of *M. incognita*, *F. oxysporum* and *R. solani* was inoculated according to the following scheme and as per procedure described earlier in the section 2.5.1, 2.6.1 and 2.7.1 respectively.

- (i) Uninoculated control
- (ii) 5000 J₂ of *M. incognita* alone
- (iii) 7.5×10⁶ cfu of *F. oxysporum* alone
- (iv) 7.5 g mycelium of *R. solani* alone
- (v) *R. solani* and *F. oxysporum* simultaneously
- (vi) *M. incognita* and *F. oxysporum* simultaneously
- (vii) *M. incognita* and *R. solani* simultaneously
- (viii) *M. incognita*, *F. oxysporum* and *R. solani* simultaneously
- (ix) *M. incognita* one week prior to *F. oxysporum*
- (x) *M. incognita* one week prior to *R. solani*
- (xi) *M. incognita* one week prior to *F. oxysporum* and *R. solani*
- (xii) *F. oxysporum* one week prior to *M. incognita*
- (xiii) *R. solani* one week prior to *M. incognita*
- (xiv) *F. oxysporum* and *R. solani* one week prior to *M. incognita*

3.2. Recording of Data

Recording of data regarding the plant growth and fruit yield, estimation of nematode population from soil and roots, root-knot index and percent root infection was done in the manner as mentioned earlier in the section 2.5.2.1, 1.3.1, 1.3.2, 1.6.2, 1.5 and 1.7 respectively.

4. In-vitro, evaluation of biocontrol agents, organic amendments and pesticides for their efficacy against *F. oxysporum* and *R. solani* alone

4.1. Screening of *T. harzianum* and *T. virens* isolates against *F. oxysporum* and *R. solani*

Antagonistic activity of isolates of *T. harzianum* and *T. virens* against *F. oxysporum* and *R. solani* were determined separately by dual culture plate technique (Morton and Stroube, 1955) to identify the most effective isolate. 5 mm diameter disc of actively growing 7 days old cultures of each test pathogen were cut with the help of sterile cork borer and placed 5 mm apart from periphery of separate petriplate containing PDA and same diameter disc of actively growing 7 days old culture of each isolate of *T. harzianum* and *T. virens* placed separately on opposite side in each petriplate. Each treatment having 5 replicates. Five petriplates inoculated with both the pathogens alone separately and without inoculation of antagonists, served as control. Later on petriplates were kept in a BOD incubator at $27 \pm 1^\circ\text{C}$. Radial growth of the pathogens in dual culture were recorded separately after 7 days of incubation for each replicate by the standard method (Brown, 1923) and the mycelial inhibition of the pathogens over control was calculated separately for isolates of both the antagonists.

4.2. Screening of the *P. fluorescens* isolates against *F. oxysporum* and *R. solani*

For determining the potential isolate of *P. fluorescens* against both the test pathogens, experiment was carried out by using dual culture plate technique. 5 mm mycelial disc of actively growing 7 days old cultures of each test pathogen was cut with the help of sterile cork borer and placed centrally in separate petriplates containing sterilized PDA. Spot application of 24 h old culture of each isolate of *P. fluorescens* was inoculated separately at four corners at 5 mm apart from the periphery of the petriplates earlier inoculated with test pathogens separately. The fungal pathogens inoculated centrally on separate PDA plates, but not inoculated with *P. fluorescens*, served as control. There were 5 replicates for each treatment. Inoculated plates were incubated at $28 \pm 1^\circ\text{C}$ for 7 days, thereafter observations in respect of radial growth of the pathogens in dual culture were recorded separately for each replicate by the standard method (Brown, 1923). The mycelial inhibition of the pathogen over control was calculated separately for each isolate of *P. fluorescens*.

4.3. Efficacy of organic amendments and pesticides against *F. oxysporum* and *R. solani*

The efficacy of organic additives (neem (*Azadirachta indica*) seed powder and farmyard manure) and pesticides (carbofuran, topsin-M and bavistin) was tested *in vitro* against *F. oxysporum* and *R. solani* by the 'poisoned food technique' (Schmitz, 1930). Neem seed powder @ 50 mg/l, farmyard manure @ 1500 mg/l, carbofuran, topsin-M and bavistin @ 1 mg a.i./l. were mixed separately in PDA medium and autoclaved for 30 min at 1 kg/cm² pressure and poured in sterilized petriplates. Each treatment replicated five times and five petriplates having untreated medium were prepared to serve as control. Later on 5 mm disc of fully grown culture of each test pathogen inoculated separately in the center of petriplate with the help of sterilized cork borer and incubated at 27 ± 1°C. Radial growth of the test fungi were measured after 7 days of incubation.

5. Comparative efficacy of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* alone and in combinations, on tomato cv. K-25 under pot conditions

5.1. Maintenance and mass production of biocontrol agents

5.1.1. *T. harzianum*

Mass culture of *T. harzianum* was maintained on PDB in 250 ml Erlenmeyer flasks. Each flask containing 100 ml PDB were plugged with cotton and sterilized by autoclaving for 30 min at 1 kg/cm² pressure. The flasks were then allowed to cool at room temperature, afterwards each flask was inoculated separately with 1-cm-diameter PDA discs punched from the periphery of actively growing 5-days-old culture of *T. harzianum* isolate TH-H-3. Flasks were then placed in a BOD incubator at 27 ± 1°C, and the fungus was allowed to grow for 10 days and after that talc was mixed in culture to maintain the 10¹⁰ cfu/g

5.1.2. *T. virens*

T. virens isolate TV-K-3 was also multiplied in the same manner as described in the section 5.1.1

5.1.3. *P. fluorescens*

The culture tubes each containing 10 ml King's 'B' broth (King *et al* , 1954) were autoclaved for 30 min at 1 kg/cm² pressure. When culture tubes were cooled, each tube was inoculated with the single colony of *P. fluorescens* isolate PS-4 from pure bacterial

culture maintained on King's 'B' medium. The culture tubes were then placed in a BOD incubator for 48 h at $30 \pm 1^\circ\text{C}$ for the multiplication of *P. fluorescens*. For mass production, 250 ml Erlenmeyer flasks containing 100 ml King's 'B' broth were autoclaved at the same pressure and time as mentioned above. Later, flasks were inoculated with 1.0 ml of *P. fluorescens* cultured broth. The flasks were then kept at $30 \pm 1^\circ\text{C}$ in a BOD incubator for 48 h and were shaken twice a day so as to get a uniform growth. Culture was then mixed with talc in the ratio of 1:4, and afterwards the amount of talc was adjusted so that the final cfu of *P. fluorescens* was maintained on $10^{10}/\text{g}$.

5.2. Application of treatments

For determining the effect of various management components, each treatment materials were mixed separately with the autoclaved 5 kg soil and farmyard manure (4:1) mixture already filled in earthen pots. The rate of application of various treatment materials is mentioned below:

Treatment	Rate of application	
	kg / ha	mg / kg soil
<i>Trichoderma harzianum</i> (Th) ($0.5 \times 10^6 \text{cfu/g soil}$)	100.0	50.0
<i>T. virens</i> (Tv) ($0.5 \times 10^6 \text{cfu/g soil}$)	100.0	50.0
<i>Pseudomonas fluorescens</i> (Pf) ($0.5 \times 10^6 \text{cfu/g soil}$)	100.0	50.0
Farm yard manure (FYM)	3000.0	1500.0
Neem seed powder (NSP)	500.0	250.0
Carbofuran (Cf) (@ 2 kg a.i./ha)	66.7	33.4
Topsin-M (T-M) (@ 2 kg a.i./ha)	2.8	1.4
Bavistin (Bv) (@ 2 kg a.i./ha)	4.0	2.0

The neem seed powder, farmyard manure and biocontrol agents were applied a week before transplantation, while pesticides were applied a day before transplantation. The treated pots were irrigated as per the need to maintain good soil moisture.

5.3. Transplantation and inoculation

The transplantation and inoculation of seedlings with *M. incognita*, *F. oxysporum*, *R. solani* alone, *F. oxysporum* + *R. solani*, *F. oxysporum* + *M. incognita*, *R. solani* + *M.*

incognita and *M. incognita* + *F. oxysporum* + *R. solani* simultaneously was done in the same manner as described in section 2.5.1, 2.6.1 and 2.7.1 respectively. Five inoculated pots for each treatment were left untreated and five pots were left uninoculated which served as control. The experiment was laid out in RBD.

5.4. Recording of data

Recording of data was done in the same manner as described in the section 2.5.2.1 for plant growth and fruit yield, section 1.3.1, 1.3.2 and 1.6.2 for nematode population in soil and roots, section 1.5 for root-knot index and section 1.7 for fungal infection in roots respectively.

6. Compatibility test, *in-vitro*, among fungal and bacterial biocontrol agents and thereof with pesticides and organic amendments

6.1. Compatibility of *T. harzianum* and *T. virens* with *P. fluorescens*

Sterilized petriplates containing PDA medium were inoculated with PS-4 isolate of *P. fluorescens* and incubated in a BOD incubator at $28 \pm 1^\circ\text{C}$ for 24 h. Later on, 5 mm disc of *T. harzianum* isolate TH-H-3 and *T. virens* isolate TV-K-3 were inoculated separately in the centre of *P. fluorescens* inoculated plates. There were five replicates for each treatment. Five plates without *P. fluorescens* and inoculated with *T. harzianum* and *T. virens* separately, served as control. Seven days after incubation, observations in respect of diameter of mycelial growth were recorded.

6.2. Compatibility of biocontrol agents with organic amendments and pesticides

Compatibility test of *T. harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 on PDA and *P. fluorescens* isolate PS-4 on King's 'B' medium with organic additives and pesticides *in vitro* was done by 'poisoned food technique' (Schmitz, 1930) as described in section 4.3. Fungal biocontrol agents were inoculated separately in the center of each petriplates containing PDA, while *P. fluorescens* was inoculated in the center of petriplates containing King's 'B' medium. Each treatment replicated five times and five plates without any treatments and inoculated with the biocontrol agents separately served as control. Seven days after inoculation, data were recorded in terms of fungal and bacterial growth.

7. Efficacy of biocontrol agents, organic amendments and pesticides alone and in combination, against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in nursery under sick plot conditions

7.1. Preparation and maintenance of sick field

Sick field of *M. incognita*, *F. oxysporum* and *R. solani* having sandy loam soil (70% sand, 22% silt, 8% clay, pH 7.5) was available in the Department of Plant Protection, Faculty of Agricultural Sciences, AMU, Aligarh. For stimulating the activities of pathogens in sick field, seedlings of a local susceptible variety of tomato were transplanted at a distance of 25x25 cm. The crop was allowed to grow 100 days before launching the experiment in the same plot. Initial population of *M. incognita* and percent root infection on plants by *F. oxysporum* and *R. solani* was taken before conducting the experiment.

7.2. Preparation of nursery beds and application of treatments

The infested field was ploughed and after leveling, nursery beds of 50x50 cm² made with 50 cm distance between nursery beds. Biocontrol agents and organic additives were applied manually 7 days before seed sowing while pesticides applied in nursery bed at the time of sowing. There were five replicates for each treatment. The experiment was laid out in RBD for single treatments and major combinations as given in table 7.2.1 to 7.2.4. The rate of application of different treatments was done according to the schemes mentioned below:

7.2.1. Application of various treatments alone

Treatments	Rate of application	
	kg per ha	g per bed
Untreated control	—	—
Th (10 ⁶ cfu/g soil)	100	2.5
Tv (10 ⁶ cfu/g soil)	100	2.5
Pf (10 ⁶ cfu/g soil)	100	2.5
FYM	3000	75.0

NSP	500	12.5
Cf	66	1.7
T-M	2.7	0.07
Bv	4.0	0.1

7.2.2. *T. harzianum* in combinations with various additives

Treatments	Rate of application	
	kg per ha	g per bed
Untreated control	–	–
Th + Pf	50 + 50	1.25 + 1.25
Th + FYM	50 + 1500	1.25 + 37.5
Th + NSP	50 + 250	1.25 + 6.25
Th + Cf	50 + 33	1.25 + 0.83
Th + Pf + FYM	33 + 33 + 1000	0.83 + 0.83 + 25
Th + Pf + NSP	33 + 33 + 167	0.83 + 0.83 + 4.18
Th + FYM + NSP	33 + 1000 + 167	0.83 + 25 + 4.18
Th + FYM + Cf	33 + 1000 + 22	0.83 + 25 + 0.55
Th + NSP + Cf	33 + 167 + 22	0.83 + 4.18 + 0.55
Th + FYM + NSP + Cf	25 + 750 + 125 + 16.5	0.63 + 18.75 + 3.13 + 0.41
Th + Pf + FYM + NSP	25 + 25 + 750 + 125	0.63 + 0.63 + 18.75 + 3.13

*In all combined treatments, rate of application was reduced to half (for integration of two components), one third (for integration of three components) and one fourth (for integration of four components) of standard rate as given in Table 7.2.1.

7.2.3. *T. virens* in combinations with various additives

Treatments	Rate of application	
	kg per ha	g per bed
Untreated control	–	–
Tv + Pf	50 + 50	1.25 + 1.25

Tv + FYM	50 + 1500	1.25 + 37.5
Tv + NSP	50 + 250	1.25 + 6.25
Tv + Cf	50 + 33	1.25 + 0.83
Tv + Pf + FYM	33 + 33 + 1000	0.83 + 0.83 + 25
Tv + Pf + NSP	33 + 33 + 167	0.83 + 0.83 + 4.18
Tv + FYM + NSP	33 + 1000 + 167	0.83 + 25 + 4.18
Tv + FYM + Cf	33 + 1000 + 22	0.83 + 25 + 0.55
Tv + NSP + Cf	33 + 167 + 22	0.83 + 4.18 + 0.55
Tv + FYM + NSP + Cf	25 + 750 + 125 + 16.5	0.63 + 18.75 + 3.13 + 0.41
Tv + Pf + FYM + NSP	25 + 25 + 750 + 125	0.63 + 0.63 + 18.75 + 3.13

*In all combined treatments, rate of application was reduced to half (for integration of two components), one third (for integration of three components) and one fourth (for integration of four components) of standard rate as given in Table 7.2.1.

7.2.4. *P. fluorescens* in combinations with various additives

Treatments	Rate of application	
	kg per ha	g per bed
Untreated control	—	—
Pf + Th	50 + 50	1.25 + 1.25
Pf + Tv	50 + 50	1.25 + 1.25
Pf + FYM	50 + 1500	1.25 + 37.5
Pf + NSP	50 + 250	1.25 + 6.25
Pf + Cf	50 + 33	1.25 + 0.83
Pf + T-M	50 + 1.35	1.25 + 0.034
Pf + Bv	50 + 2.0	1.25 + 0.05
Pf + Th + FYM	33 + 33 + 1000	0.83 + 0.83 + 25
Pf + Th + NSP	33 + 33 + 167	0.83 + 0.83 + 4.18
Pf + Tv + FYM	33 + 33 + 1000	0.83 + 0.83 + 25
Pf + Tv + NSP	33 + 33 + 167	0.83 + 0.83 + 4.18

Pf + FYM + NSP	33 + 1000 + 167	0.83 + 25 + 4.18
Pf + FYM + Cf	33 + 1000 + 22	0.83 + 25 + 0.55
Pf + NSP + Cf	33 + 167 + 22	0.83 + 4.18 + 0.55
Pf + FYM + T-M	33 + 1000 + 0.9	0.83 + 25 + 0.02
Pf + NSP + T-M	33 + 167 + 0.9	0.83 + 4.18 + 0.02
Pf + FYM + Bv	33 + 1000 + 1.33	0.83 + 25 + 0.03
Pf + NSP + Bv	33 + 167 + 1.33	0.83 + 4.18 + 0.03
Pf + Th + FYM + NSP	25 + 25 + 750 + 125	0.63 + 0.63 + 18.75 + 3.13
Pf + Tv + FYM + NSP	25 + 25 + 750 + 125	0.63 + 0.63 + 18.75 + 3.13
Pf + FYM + NSP + Cf	25 + 750 + 125 + 16.5	0.63 + 18.75 + 3.13 + 0.41
Pf + FYM + NSP + T-M	25 + 750 + 125 + 0.68	0.63 + 18.75 + 3.13 + 0.02
Pf + FYM + NSP + Bv	25 + 750 + 125 + 1.00	0.63 + 18.75 + 3.13 + 0.03

*In all combined treatments, rate of application was reduced to half (for integration of two components), one third (for integration of three components) and one fourth (for integration of four components) of standard rate as given in Table 7.2.1.

7.3. Sowing of tomato seeds

As described earlier (section 7.2) 100 seeds of tomato cv. K-25 were sown in each bed separately as per schemes mentioned above. Irrigation was done with the help of sprinkler as per the requirement.

7.4. Recording of data

7.4.1. Number and fresh weight of seedlings

Recording of data regarding the number and fresh weight of total seedlings per bed was done 45 days after sowing.

7.4.2. Root-knot index and percent root infection

Data regarding the root-knot index and percent root infection was recorded in the same manner as mentioned in section 1.5 and 1.7 respectively.

8. Evaluation of various treatments of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in sick field conditions

8.1. Preparation of plots in sick field and application of various treatments

After ploughing of sick field, experimental plots of 3.75 x 3 m² size were prepared. The application of various treatment materials of selected treatments was done according to the scheme mentioned below:

Treatments	Rate of application	
	kg / ha	g / plot
Untreated Control	—	—
Th + Pf + FYM + NSP	25 + 25 + 750 + 125	28 + 28 + 844 + 70
Tv + Pf + FYM + NSP	25 + 25 + 750 + 125	28 + 28 + 844 + 70
Th + FYM + NSP + Cf	25 + 750 + 125 + 16.5	28 + 844 + 70 + 19
Tv + FYM + NSP + Cf	25 + 750 + 125 + 16.5	28 + 844 + 70 + 19
Pf + FYM + NSP + Cf	25 + 750 + 125 + 16.5	28 + 844 + 70 + 19
Pf + FYM + NSP + T-M	25 + 750 + 125 + 0.68	28 + 844 + 70 + 0.77
Pf + FYM + NSP + Bv	25 + 750 + 125 + 1.00	28 + 844 + 70 + 1.13
Th + FYM + Cf	33 + 1000 + 22	37 + 1125 + 25
Th + Pf + NSP	33 + 33 + 167	37 + 37 + 188
Th + NSP + Cf	33 + 167 + 22	37 + 188 + 25
Tv + NSP + Cf	33 + 167 + 22	37 + 188 + 25
Pf + NSP + Cf	33 + 167 + 22	37 + 188 + 25
Pf + NSP + T-M	33 + 167 + 0.9	37 + 188 + 1.00
Pf + NSP + Bv	33 + 167 + 1.33	37 + 188 + 1.50

*In all combined treatments, rate of application was reduced to half (for integration of two components) and one fourth (for integration of four components) of standard rate as given in Table 7.2.1.

8.2. Transplantation of seedlings

45-days-old seedlings were transplanted into each plot at 75 x 60 cm distance. The number of seedlings (25 seedlings/plot) per row and distance were equal in all the treatments. There were five replicates for each treatment and five plots were left untreated which served as control. Irrigation was done as per the requirement. The experiment was laid out in completely randomized block design.

8.3. Recording of data

8.3.1. Plant growth and fruit yield

Data regarding the fruit yield was recorded during 95 days to 120 days after transplantation. Fruit weight per plot was taken from each treatment and replicate separately. Thereafter, fresh weight of plants per plot was determined. Dry weight was recorded in the manner as described in section 2.5.2.1.

8.3.2. Estimation of root-knot nematode population in soil and roots, root-knot index and percent root infection by fungi was done in the same manner as described in section 1.3.1, 1.3.2, 1.6.2, 1.5 and 1.7 respectively.

9. Statistical analyses

For determining the significance of the treatments, the data were subjected to statistical analyses. The experiments were laid down using the pattern of RBD. The data were statistically analyzed by analyses of variance (ANOVA) as suggested by Cochran and Cox (1957). Significant differences were determined by using critical difference (CD) test at 5% and 1% probability level.

CHAPTER – 4

RESULTS

1. Survey for the occurrence, distribution and identification of nematodes and fungi, causing disease complex in tomato, and for collection of naturally occurring fungal and bacterial antagonists from western districts of Uttar Pradesh

Survey of farmer's fields growing tomato was conducted at different localities of Agra, Aligarh, Bulandshahar and Mathura districts of western Uttar Pradesh during the month of April 2004 and March 2005. During both the surveys, almost all the stages of crop growth were considered for observation. Observation regarding the above and underground symptoms, extent of infection caused by nematodes and fungi, isolation and identification of plant parasitic nematodes from the roots and rhizosphere soil, pathogenic fungi infecting roots, and isolation and identification of biocontrol agents especially *Trichoderma* species and *Pseudomonas fluorescens* from the rhizosphere soil of tomato was done during the survey are presented below.

1.1 Symptomatology

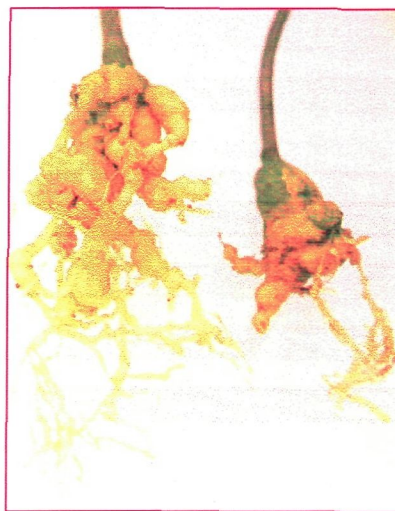
Visual observations indicated that the patchy appearance with yellowing of leaves, chlorosis, stunting and wilting was commonly found in most of the localities. Collar rot with wire stem symptom was also observed at nursery and early stages of plant growth in most of the localities (Plate 1 A, B). In some localities, the crop was severely affected with root-knot-wilt-root-rot disease complex showing yellowish brown leaves, stunted plant growth and most of the plants were died due to wilting with wire stem symptoms having brown colour stem at collar region. These plants had less fruits and poor growth (Plate 2 A, B).

Infected plants showing diseased symptoms were uprooted carefully and observation was made in the laboratory. Studies indicated that in most of the localities low to severe galling along with fungal infection was frequently observed on root system [Plate 2 B (3)]. In early stages of plant growth, symptoms of wire stem, collar rot or root-rot along with galling of root systems were also observed [Plate 1 B (3)]. However, in

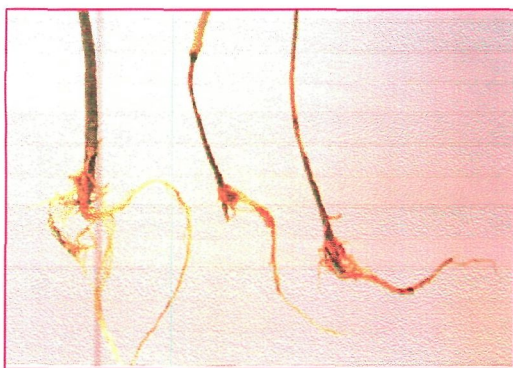
A



**B
(1)**



B (2)



B (3)



Plate 1: A view of farmer's field showing disease symptoms on tomato seedlings in nursery beds

- (A) Nursery beds showing above ground symptoms of wilting, collar rot with stunting growth of seedlings
(B) Showing underground symptoms (1) galled root (2) wilt, collar rot, root rot and wire stem (3) root-knot-wilt-root rot

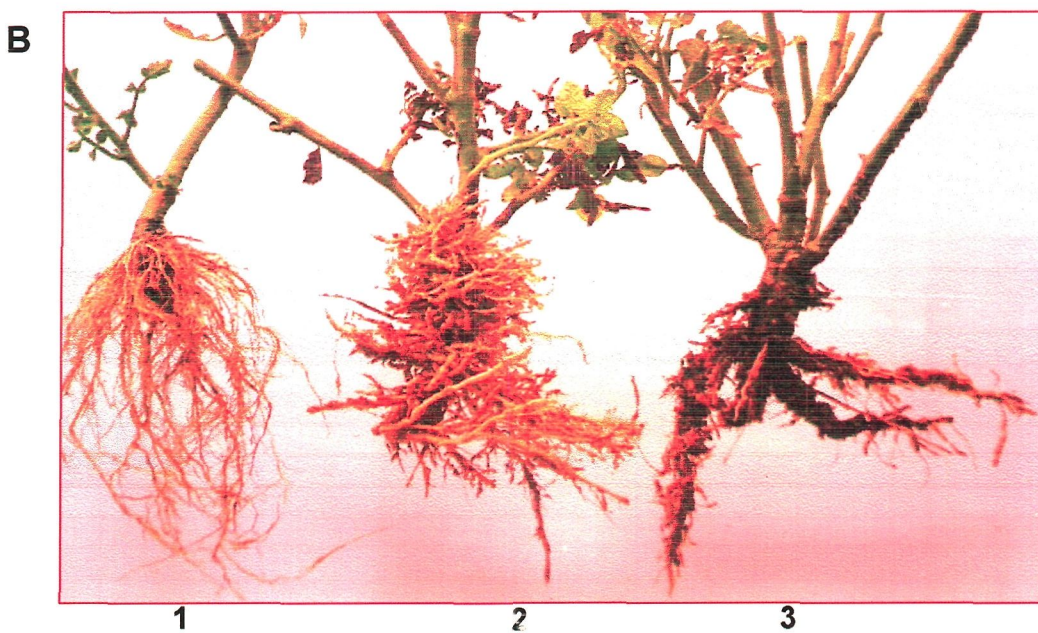


Plate 2: A view of farmer's field showing disease symptoms on tomato plants (A) symptoms on roots (B)

1. Uninfected root
2. Showing root galls caused by *Meloidogyne incognita*
3. Showing root galls caused by *Meloidogyne incognita* along with rotting and blackening of roots due to infection of *Fusarium oxysporum* and *Rhizoctonia solani*

some localities, where infection was severe, the roots were found rotted and plants became died.

1.2 Plant parasitic nematodes in the rhizosphere soil

Data (Table 1 and 2) regarding the population of plant parasitic nematodes in the soil samples collected from rhizosphere of tomato grown in different localities of Agra, Aligarh, Bulandshahar and Mathura districts of Uttar Pradesh during April 2004 and March 2005 observed between the range of 0 to 7300/250 g soil. Nematode population isolated during both the surveys composed of *Helicotylenchus* spp., *Hoplolaimus* spp., larvae of *Meloidogyne* spp., *Rotylenchulus* sp., *Tylenchorhynchus* spp. and *Xiphinema* spp. In some samples *Criconimoides* spp., *Heterodera* spp., *Longidorus* spp., *Pratylenchus* spp. and *Tylenchus* spp. were also found occasionally. Among all the genera, *Tylenchorhynchus* was dominated the population followed by *Meloidogyne*, *Hoplolaimus*, *Rotylenchulus*, *Helicotylenchus* and *Xiphinema*, respectively.

1.2.1 Average percent occurrence of nematodes

Data (Table 3 and 4) regarding the average percent occurrence (total number of sample basis) of different genera of plant parasitic nematodes in the area under study indicated that *Tylenchorhynchus* spp. (94.2%) was most frequently distributed nematode followed by *Meloidogyne* spp. (78.5%), *Hoplolaimus* spp. (53.4%), *Rotylenchulus* sp. (42.8%), *Helicotylenchus* spp. (39.6%) and *Xiphinema* spp. (33.9%), respectively. However, percent occurrence of these nematodes varied from district to district.

In Agra, *Tylenchorhynchus* spp. was present in 92.9% of the localities followed by *Meloidogyne* spp. (63.1%), *Hoplolaimus* spp. (50.0%), *Xiphinema* spp. (29.8%), *Helicotylenchus* spp. (26.2%) and *Rotylenchulus* sp. (23.8%), respectively.

In Aligarh, *Meloidogyne* spp. was found to occur in all the localities (100%) followed by *Tylenchorhynchus* spp. (90.3%), *Rotylenchulus* sp. (75.7%), *Hoplolaimus* spp. (58.8%), *Xiphinema* spp. (36.7%) and *Helicotylenchus* spp. (31.2%), respectively.

In Bulandshahar, *Tylenchorhynchus* spp. was dominated in 97.1% of the localities followed by *Hoplolaimus* spp. (91.2%), *Meloidogyne* spp. (72.4%), *Helicotylenchus* spp. (65.3%), *Xiphinema* spp. (62.4%) and *Rotylenchulus* sp. (34.7%), respectively.

Table 1: The occurrence of plant parasitic nematodes and soil borne fungi associated with tomato during April 2004 (Survey I)

Districts/ Localities	Nematode population/ 250 g soil	Percent occurrence							<i>Meloidogyne</i> spp./g roots	^b Root- knot index	'Percent root infection
		<i>Meloidogyne</i> spp.	<i>Tylenchorhynchus</i> spp.	<i>Hoplotaimus</i> spp.	<i>Helicotylenchus</i> spp.	<i>Rotylenchulus</i> sp.	<i>Xiphinema</i> spp.	Others ^a			
Agra											
Baralgusa	1500	20	80	-	-	-	-	-	160	1.75	50
Gopau	900	-	55	-	22.2	-	-	22.2	-	-	40
Govindpur	400	-	50	-	25	-	25	-	-	-	70
Hasanpur	500	20	40	20	-	20	-	-	50	0.75	80
Lahlar	600	33.3	50	-	-	-	16.7	-	40	0.5	85
Lodhenagla	250	-	-	-	-	-	100	-	-	-	52
Midhakur	300	-	33.3	33.3	-	-	-	-	-	-	58
Pathouli	2200	4.5	4.5	72.7	-	-	-	18.3	275	2.25	60
Pripokhar	1900	-	47.4	26.3	10.5	-	15.8	-	-	-	63
Rohankala	1100	-	72.7	9.1	-	-	9.1	9.1	-	-	15
Ramnagar	900	-	-	22.2	-	-	66.7	11.1	-	-	15
Sahla	2600	7.7	7.7	3.8	-	69.3	-	11.5	300	2.5	65
Satera	600	-	83.3	-	-	-	-	16.7	-	-	70
Ujarai	1000	30	20	-	10	-	-	40	120	1.25	70
Aligarh											
Baroula	500	20	40	-	-	40	-	-	70	1.2	20
Bhukrawali	700	42.9	-	14.3	28.6	14.3	-	-	200	2.25	55
Gangagarh	1000	10	10	20	30	-	10	20	100	1.35	72
Gjrouli	1700	47	23.5	-	-	17.7	5.9	5.9	1250	4	25
Haridaspur	2100	4.8	66.7	7.1	-	19	-	2.4	150	2	67
Iglas	3700	70.3	21.6	-	-	5.4	-	2.7	1375	4	43
Jalalpur	800	12.5	18.8	6.2	-	62.5	-	-	120	1.35	76
Jawan	1100	9.1	18.2	45.4	-	-	18.2	9.1	80	1.15	80
Malaypur	550	20	-	-	20	60	-	-	40	0.55	91
Murari Nagla	400	25	50	-	-	25	-	-	80	1.1	78
Nagla kila	1000	20	30	-	40	5	5	-	300	2.75	69
Nagla Kunjalpur	1200	16.7	50	-	-	33.3	-	-	450	3.25	59
Nagla Sumali	1000	10	10	45	20	-	10	5	100	1.15	82
Nangalia	500	20	60	-	-	20	-	-	80	1	75
Pilakhuni	400	25	50	-	-	25	-	-	50	0.7	70
Ramgarh Panjpur	2000	7.5	65	7.5	2.5	15	-	2.5	220	2.45	62
Sarsaul	1600	6.2	6.2	68.8	-	18.8	-	-	200	2.37	73
Sasni	1400	7.1	42.9	35.8	7.1	-	7.1	-	250	2.75	51
Shahjamal	2200	20.5	45.4	-	-	27.3	-	6.8	460	3.15	40

Table 2: The occurrence of plant parasitic nematodes and soil borne fungi associated with tomato during March 2005 (Survey II)

Districts/ Localities	Nematode population/ 250 g soil	Percent occurrence							<i>Meloidogyne</i> spp./g roots	^b Root- knot index	^c Percent root infection
		<i>Meloidogyne</i> spp.	<i>Tylenchorhynchus</i> spp.	<i>Hoplolaimus</i> spp.	<i>Helicotylenchus</i> spp.	<i>Rotylenchulus</i> sp.	<i>Xiphinema</i> spp.	Others ^a			
Agra											
Hasanpur	400	12.5	50	25	-	12.5	-	-	40	0.5	75
Lahlar	550	30	50	-	-	-	20	-	30	0.5	75
Pathouli	2000	2.5	2.5	75	-	-	-	20	200	2	60
Sahta	2400	6.25	6.25	4.2	-	66.7	-	16.7	220	2	65
Satera	600	-	66.7	-	-	-	-	33.3	-	-	60
Ujarai	800	31.25	25	-	12.5	-	-	31.25	80	1	60
Aligarh											
Gijrouli	1500	40	23.3	16.7	-	3.3	6.7	10	1000	4	40
Iglas	4000	62.5	-	20	-	3.75	-	13.75	1300	4	30
Jalapur	600	8.3	16.7	8.3	-	66.7	-	-	50	0.83	70
Murari nagla	400	12.5	25	12.5	-	37.5	-	12.5	90	0.9	80
Nagla kila	900	16.7	22.2	-	-	38.9	-	11.1	200	2.2	70
Nagla kunjapur	1200	16.7	41.7	-	4.2	25	4.2	8.3	220	2.6	65
Ramgarh panjipur	1800	5.5	69.4	5.5	2.8	13.9	-	2.8	150	1.5	60
Sasni	1200	4.2	41.7	37.5	4.2	-	8.3	4.2	200	2.1	50
Shahjamal	2500	20	40	2	-	26	-	12	375	3.25	50
Udla iliyaspur	1000	5	60	-	-	-	15	20	50	0.95	70
Bulandshahar											
Danpur	2800	48.2	14.3	5.4	12.5	1.8	7.1	10.7	1000	4	45
Devi ka nagla	1200	-	25	33.3	41.7	-	-	-	-	-	50
Halpura	1000	45	30	10	-	-	5	10	200	2	55
Khurja	1000	25	25	10	10	25	-	5	275	2	57
Nagla chhatari	1000	40	25	15	-	-	15	5	300	2.25	60
Mathura											
Ajaynagar	1500	56.7	13.3	-	-	16.7	-	13.3	375	2.75	40
Aurangabad	2800	92.9	1.8	-	-	-	-	5.3	700	3.75	50
Gokul	1200	83.3	12.5	-	-	-	-	4.2	300	2.5	40
Karnawal	7000	80.1	7.1	-	-	5.7	-	7.1	1500	4	25
Koilalpur	300	66.7	33.3	-	-	-	-	-	220	2.25	60
Kumhan	300	-	33.3	-	33.3	-	-	33.3	-	-	50

^aOthers:- *Pratylenchus* spp., *Tylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Cricomimoides* spp.

^bRoot-knot index: 0= 0%, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4= 76-100%

^cPercent root infection by fungi

Table 3: Average percent occurrence of plant parasitic nematodes in different districts during April 2004 (Survey I)

Districts	<i>Meloidogyne</i> spp.	<i>Tylenchorhynchus</i> spp.	<i>Hoplolaimus</i> spp.	<i>Helicotylenchus</i> spp.	<i>Rotylenchulus</i> sp.	<i>Xiphinema</i> spp.	Others ^a
Agra (14) ^b	42.9	85.7	50.0	35.7	14.3	42.9	50.0
Aligarh (21)	100.0	90.5	47.6	33.3	71.7	33.3	38.1
Bulandshahar (17)	64.7	94.1	82.4	70.6	29.4	64.7	41.2
Mathura (15)	73.3	93.3	26.7	53.3	40.0	13.3	26.7
Average	70.2	90.9	51.7	48.2	38.8	38.6	39.0

^aOthers: *Pratylenchus* spp., *Tylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Criconimoides* spp.

^bFigure in parentheses are total number of samples

Table 4: Average percent occurrence of plant parasitic nematodes in different districts during March 2005 (Survey II)

Districts	<i>Meloidogyne</i> spp.	<i>Tylenchorhynchus</i> spp.	<i>Hoplolaimus</i> spp.	<i>Helicotylenchus</i> spp.	<i>Rotylenchulus</i> sp.	<i>Xiphinema</i> spp.	Others ^a
Agra (6) ^b	83.3	100.0	50.0	16.7	33.3	16.7	66.7
Aligarh (10)	100.0	90.0	70.0	30.0	80.0	40.0	90.0
Bulandshahar (5)	80.0	100.0	100.0	60.0	40.0	60.0	80.0
Mathura (6)	83.3	100.0	0.0	16.7	33.3	0.0	83.3
Average	86.7	97.5	55.0	30.9	46.7	29.2	80.0

^aOthers: *Pratylenchus* spp., *Tylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Criconimoides* spp.

^bFigure in parentheses are total number of samples

In Mathura, *Tylenchorhynchus* spp. was present in 96.7% localities followed by *Meloidogyne* spp. (78.3%), *Rotylenchulus* sp. (36.7%), *Helicotylenchus* spp. (35.0%), *Hoplolaimus* spp. (13.4%) and *Xiphinema* spp. (6.7%), respectively.

1.2.2 Average population composition and nematode population in different districts

Data regarding average percent population composition of nematodes showed that during survey I, the most dominant nematode was *Tylenchorhynchus* spp. (30.5%) followed by *Meloidogyne* spp. (21.9%), *Hoplolaimus* spp. (13.0%), *Helicotylenchus* spp. (11.4%), *Rotylenchulus* sp. (8.5%) and *Xiphinema* spp. (6.5%), respectively (Table 5; Fig. 1 A). However, during survey II, *Meloidogyne* spp. was most dominant (32.0%) followed by *Tylenchorhynchus* spp. (27.1%), *Rotylenchulus* sp. (11.0%), *Hoplolaimus* spp. (10.6%), *Helicotylenchus* spp. (5.4%) and *Xiphinema* spp. (3.0%), respectively (Table 6; Fig. 1 B).

The population of plant parasitic nematodes (per 250 g soil) was recorded to be 7300 and 7000 at Mathura followed by Aligarh (3700 and 4000), Bulandshahar (2800 and 2800) and Agra (2600 and 2400) during I and II survey, respectively (Table 5 and 6).

1.2.3 Root-knot index

Data regarding the root-knot index (RKI) during both the surveys indicated that the RKI ranged between 0.0 to 4.00 as presented in Table 1 and 2.

During I and II surveys the highest RKI (2.00 and 2.54) was found at Mathura followed by Aligarh (1.94 and 2.23), Bulandshahar (1.60 and 2.10) and Agra (0.64 and 1.00), respectively (Table 5 and 6; Fig. 2 A, B).

1.2.4 Identification of root-knot nematode species

Studies pertaining to identification of root-knot nematode species infesting tomato in different localities revealed that *M. incognita* (75%) was more prevalent than *M. javanica* (25%).

1.2.5 Population of root-knot nematodes in roots

The population of root-knot nematodes in the areas surveyed during April 2004 was between the range of 0 - 1775/g roots of tomato, while during March 2005, population was observed between the range of 0-1500/g roots (Table 1 and 2).

Table 5: Average population composition (%) of plant parasitic nematodes in the rhizosphere of tomato and percent root infection by fungi during April 2004 (Survey I)

Districts	Nematode population / 250 g soil	Nematode population (%)						<i>Meloido</i> spp. / g roots	^b Root-knot index	^c Percent root infection
		<i>Meloido</i> spp.	<i>Tylencho rhynchus</i> spp.	<i>Hoplo latius</i> spp.	<i>Helicoty lenchus</i> spp.	<i>Rotyln chulus</i> sp.	<i>Xiphi nema</i> spp.			
Agra (14) ^d	1054.0	8.3	38.9	13.4	7.2	6.4	16.7	68.0	0.64	57.4
Aligarh (21)	1207.0	22.5	33.5	12.7	10.6	18.5	3.2	274.0	1.94	63.0
Bulandshahar (17)	1288.0	17.2	26.1	22.5	12.0	5.4	5.3	269.0	1.60	47.6
Mathura (15)	1533.0	39.8	23.3	3.4	15.6	3.5	0.9	471.0	2.00	42.4
Average	1268.5	21.9	30.5	13.0	11.4	8.5	6.5	270.5	1.55	52.6

^aOthers: *Pratylenchus* spp., *Tylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Cricoinimoides* spp.

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungi

^dFigures in parentheses are total number of samples

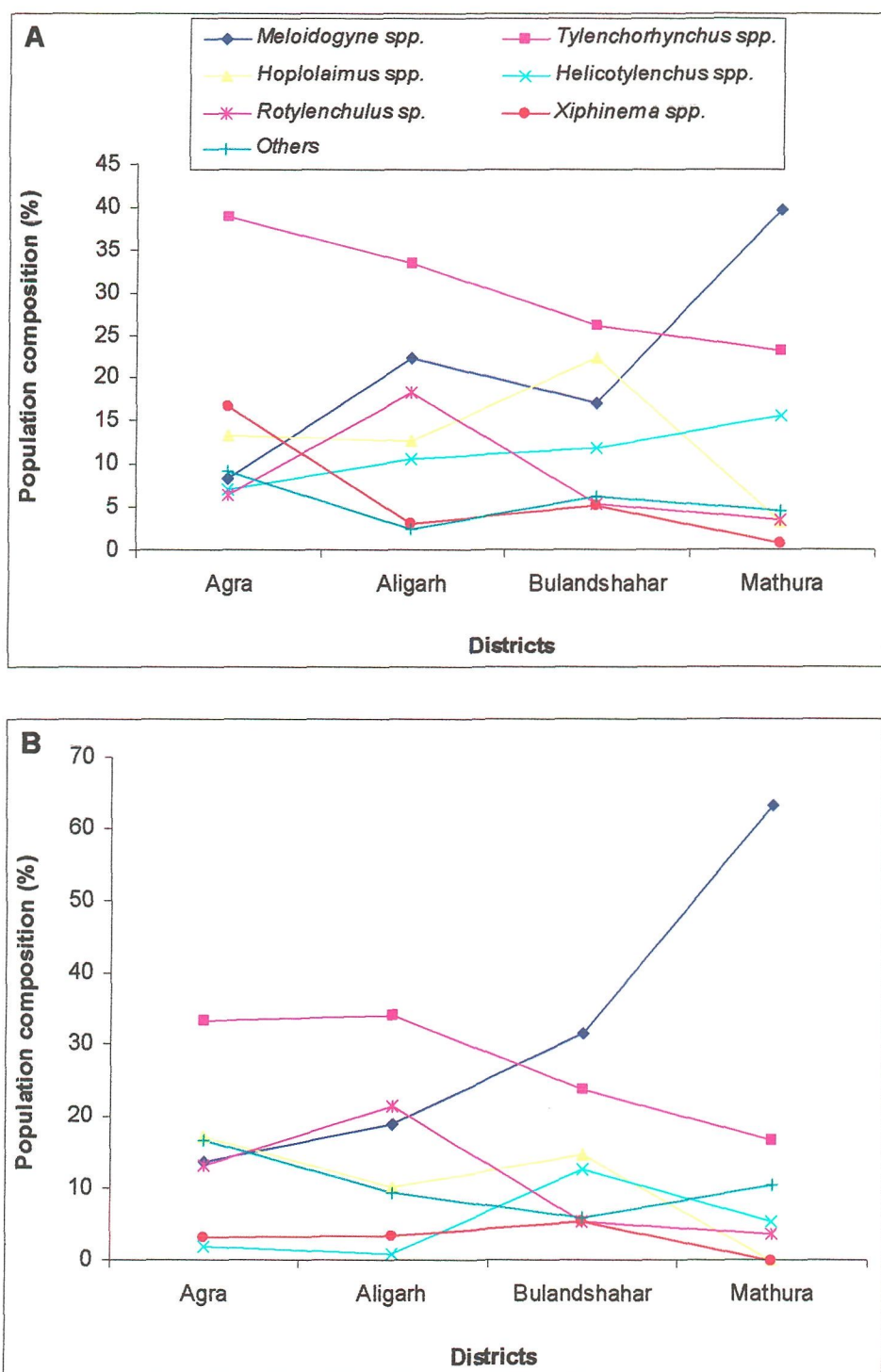


Fig. 1 : Average population composition of plant parasitic nematodes in the rhizosphere of tomato grown in various districts during April (A) 2004 March (B) 2005

Table 6: Average population composition (%) of plant parasitic nematodes in the rhizosphere of tomato and percent root infection by fungi during March 2005 (Survey II)

Districts	Nematode population (%)								<i>Metoido</i> <i>gyne</i> spp. / g roots	^b Root- knot index	^c Percent root infection
	Nematode population / 250 g soil	<i>Metoido</i> <i>gyne</i> spp.	<i>Tylencho</i> <i>rhynchus</i> spp.	<i>Hoplo</i> <i>laimus</i> spp.	<i>Helicoty</i> <i>lenchus</i> spp.	<i>Rotyln</i> <i>chulus</i> sp.	<i>Xiphi</i> <i>nema</i> spp.	Others ^a			
Agra (6) ^d	1125	13.8	33.4	17.4	2.1	13.2	3.3	16.9	95.0	1.00	65.8
Aligarh (10)	1510	19.1	34.0	10.3	1.1	21.5	3.4	9.5	364.0	2.23	58.5
Bulandshahar (5)	1400	31.6	23.9	14.7	12.8	5.4	5.4	6.1	355.0	2.10	53.4
Mathura (6)	2183	63.3	16.9	0.0	5.6	3.7	0.0	10.5	516.0	2.54	44.2
Average	1554.5	32.0	27.1	10.6	5.4	11.0	3.0	10.8	332.5	1.97	55.5

^aOthers: *Pratylenchus* spp., *Tylenchus* spp., *Heterodera* spp., *Longidorus* spp., *Criconimoides* spp.

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungi

^dFigures in parentheses are total number of samples

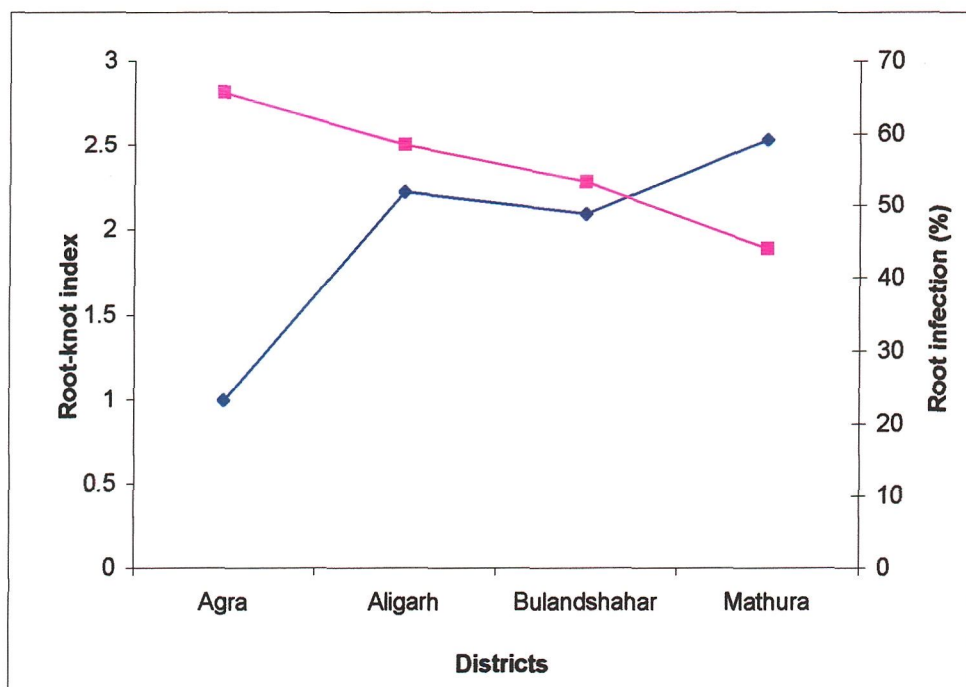
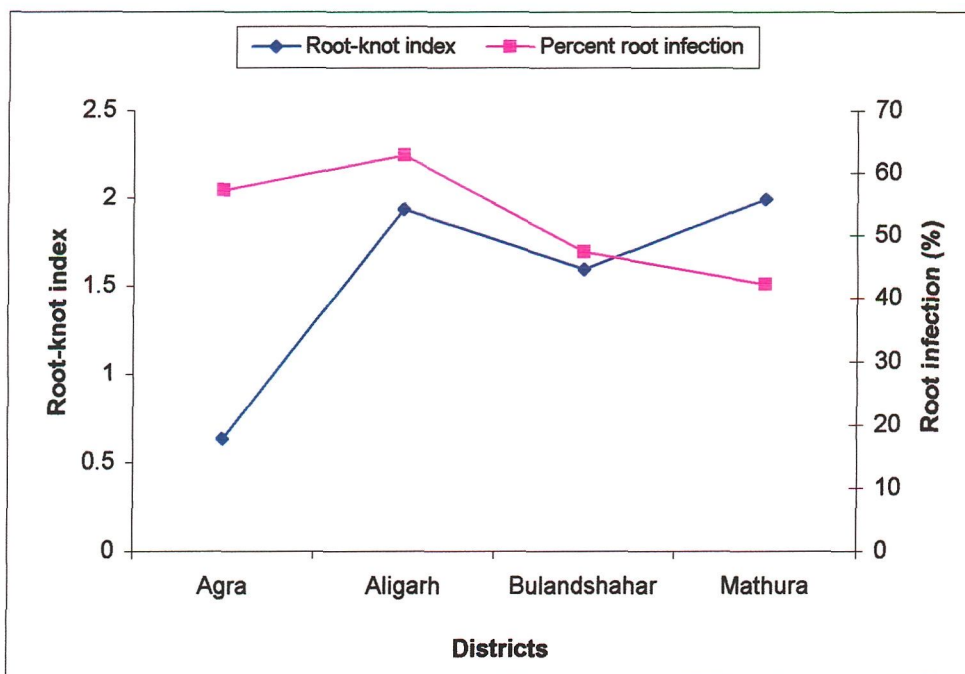


Fig. 2: Average root-knot index and percent infection by fungi in roots of tomato in various districts during April (A) 2004 and March (B) 2005

The highest population of *Meloidogyne* spp./g roots was found in Mathura (1775 and 1500) followed by Aligarh (1375 and 1300), Bulandshahar (1100 and 1000) and Agra (300 and 220) during survey I and II, respectively.

1.3 Isolation and identification of fungi infesting roots of tomato

On the basis of cultural, morphological characters and microscopic studies, various fungi isolated from the roots of diseased tomato plants were identified and further confirmed with the standard references. *Fusarium oxysporum* (Plate 3 A) and *Rhizoctonia solani* (Plate 3 B) were found to be the most frequently encountered pathogenic fungi in almost all the localities. Besides, the above fungi, *F. chlamydosporium*, *F. solani*, *Macrophomina phaseolina*, *Pythium aphanidermatum* and *P. ultimum* were also isolated from the infested roots of tomato plants.

1.4 Percent root infection by fungi

Data regarding percent root infection (PRI) of *F. oxysporum* and *R. solani* ranged between 15 to 91% and 25 to 85% during I and II survey, respectively (Table 1 and 2). Analyses of the data showed that on an average, the PRI was 52.6% during survey I, while 55.5% during survey II (Table 5 and 6).

However, the highest PRI (57.4 and 65.8%) was observed at Agra followed by Aligarh (63.0 and 58.5%), Bulandshahar (47.6 and 53.4%) and Mathura (42.4 and 44.2%) during I and II survey, respectively (Table 5, 6 and Fig. 2 A, B).

1.5 Isolation and Identification of naturally occurring fungal and bacterial bioagents

1.5.1 *Trichoderma* species

In order to identify the potential isolates of *Trichoderma* species, particularly *T. harzianum* (Plate 4 A) and *T. virens* (Plate 4 B), twelve isolates of *T. harzianum* and six isolates of *T. virens* were studied for their antagonistic potential in identified and selected for management studies. Among *T. harzianum* isolates, TH-AG-2 and TH-AG-5 yielded from Agra, whereas, isolates TH-AL, TH-H-3, TH-JP-2, TH-MN-2, TH-SP, TH-SP-1 and TH-UIP-2 from Aligarh, isolates TH-BS-6 and TH-K-9 from Bulandshahar and isolate TH-M-7 isolated from Mathura. Similarly among of *T. virens*, isolates, TV-AG-3

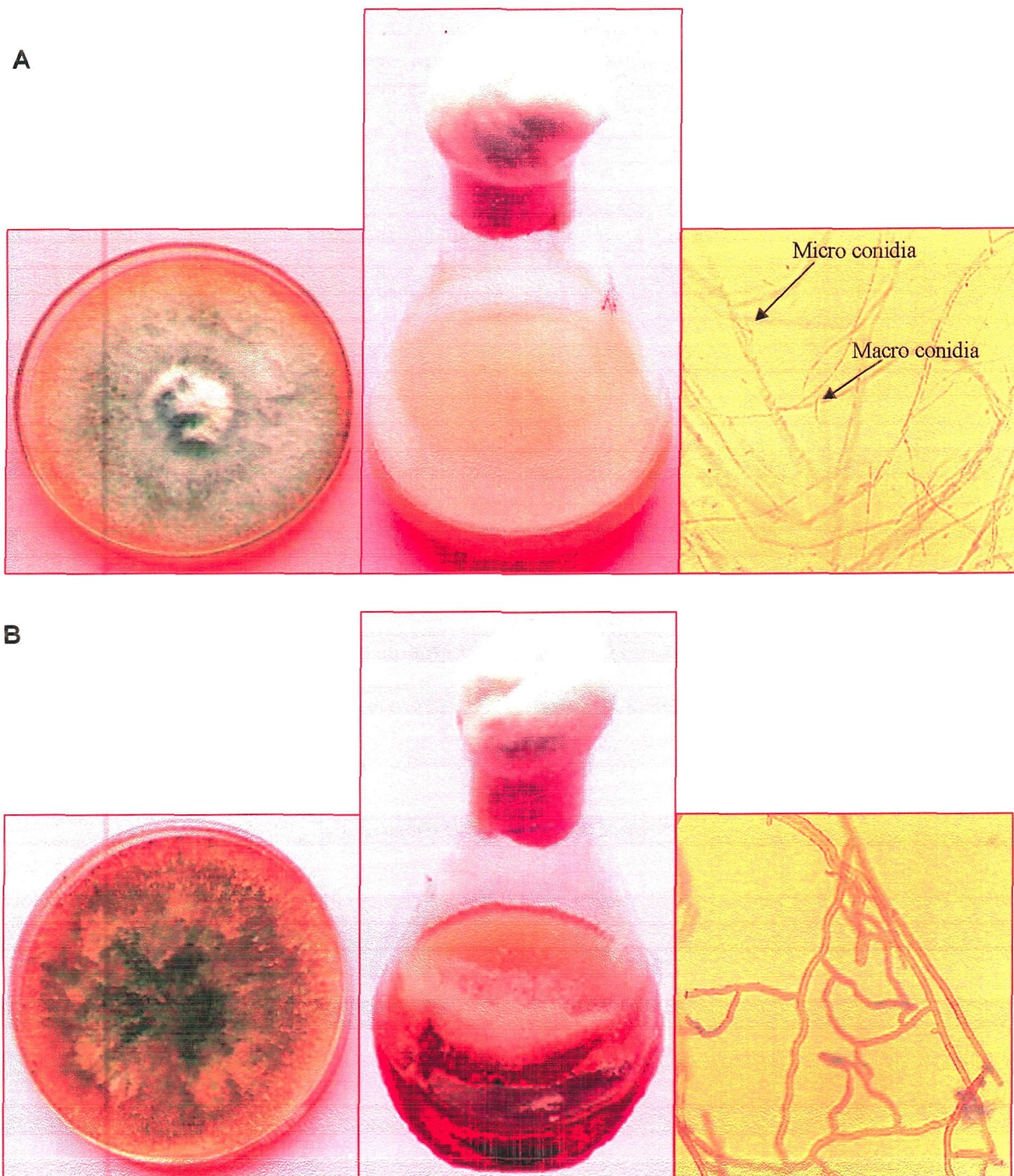


Plate 3: Cultural and morphological characteristics of wilt and root-rot causing pathogens:

(A) *Fusarium oxysporum*
 (B) *Rhizoctonia solani*

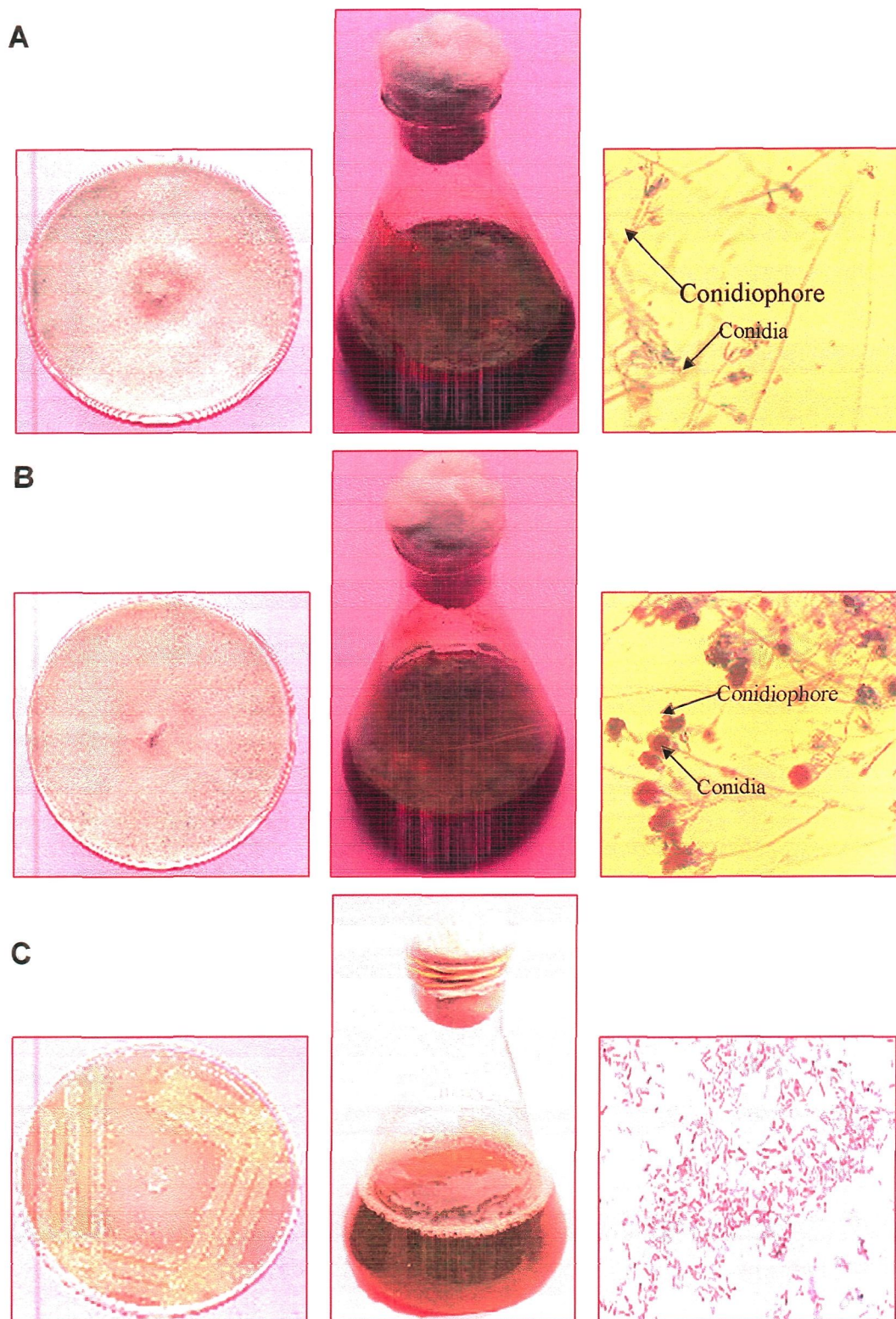


Plate 4: Cultural and morphological characteristics of biocontrol agents:

(A) *Trichoderma harzianum*
(C) *Pseudomonas fluorescens*

(B) *Trichoderma virens*

was from Agra, whereas, TV-AL-1 from Aligarh, TV-K-3 from Bulandshahar and TV-M-1 and TV-M-5 from Mathura.

1.5.2 *P. fluorescens*

Similarly, during the search of potential isolates of fluorescent *Pseudomonads* (Plate 4 C) from different districts, seventeen isolates of *P. fluorescens* were identified and selected for management studies. Among them, isolates AG-1 and AG-4 were isolated from Agra, BW-1, BW-2, H-2, NK-1, NK-2, PS-4, PS-5, PS-9, RGP-1, UIP-1 and UIP-2 from Aligarh, K-1 and K-3 from Bulandshahar and M-3 and M-5 from Mathura, respectively.

2. Pathogenicity test of *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* alone on tomato cv. K-25 under pot conditions

2.1 *M. incognita* alone

Data regarding the effect of different initial inoculum levels (Pi) of *M. incognita* on the root-knot development, plant growth and fruit yield of tomato cv. K-25, revealed that in general, reduction in various growth parameters viz., number of fruits, fruit yield, shoot-root fresh and dry weights was increased with the corresponding increase in Pi (Table 7; Fig. 3; Plate 5). However, the significant ($P \leq 0.05$) reduction in all growth parameters was found at the lowest Pi (2500 J₂/5 kg soil) as compared to uninoculated control. The highest reduction in number of fruits, fruit yield, shoot-root fresh and dry weights was 61.5, 64.2, 44.9, 55.1, 49.1 and 60.0%, respectively at the highest Pi (25,000 J₂/5 kg soil) when compared to uninoculated control (Table 7). Analyses of data indicated that differences in all the plant growth parameters among different Pi were significant ($P \leq 0.05$).

The RKI and final nematode population (Pf) in roots and soil was noted to be increased with the corresponding increase in Pi, while the reproduction factor (Rf) decreased. Highest Pf (1,23,420) was found at the highest Pi and maximum RKI (4.00) was observed at Pi 20,000 J₂ and 25,000 J₂/5 kg soil. Whereas, highest Rf (15.3) was found at the lowest Pi (Table 8; Fig. 3). Analyses of data indicated that differences in Pf, Rf and RKI among different Pi were significant ($P \leq 0.05$). However, there was no difference ($P \leq 0.05$) in RKI between Pi 20,000 and 25,000 J₂/5 kg soil (Table 8).

Table 7: Effect of different initial inoculum levels of *Meloidogyne incognita* on plant growth and fruit yield of tomato cv. K-25 under pot conditions^a

Initial Inoculum Levels (J ₂ /5 kg soil)	Number of Fruits	Fruit Weight (g)	Plant Length (cm)		Plant Fresh Weight (g)			Plant Dry Weight (g)			
			Shoot Height	Root Length	Total	Shoot	Root	Total	Shoot	Root	Total
0	10.4	223.5	46.7	28.8	75.5	175.5	37.9	213.4	37.5	11.5	49.0
2500	8.8 (15.4) ^b	183.5 (17.9)	42.1 (9.9)	25.5 (11.5)	67.6 (10.5)	154.7 (11.9)	32.7 (13.7)	187.4 (12.2)	32.3 (13.9)	9.7 (15.7)	42.0 (14.3)
5000	7.8 (25.0)	160.0 (28.4)	38.5 (17.6)	23.3 (19.1)	61.8 (18.1)	140.9 (19.7)	29.7 (21.6)	170.6 (20.1)	29.1 (22.4)	8.7 (24.3)	37.8 (22.9)
10000	7.0 (32.7)	142.5 (36.2)	36.3 (22.3)	21.1 (26.7)	57.4 (24.0)	128.7 (26.7)	26.7 (29.6)	155.4 (27.2)	26.3 (29.3)	7.7 (33.0)	34.0 (30.6)
15000	6.2 (40.4)	125.0 (44.1)	33.7 (27.8)	19.1 (33.7)	52.8 (30.1)	118.5 (32.5)	23.5 (38.0)	142.0 (33.5)	24.0 (36.0)	6.7 (41.7)	30.7 (37.3)
20000	5.2 (50.0)	105.0 (53.0)	30.9 (33.8)	17.3 (39.9)	48.2 (36.2)	107.0 (39.0)	20.0 (47.2)	127.0 (40.5)	21.5 (42.7)	5.6 (51.3)	27.1 (44.7)
25000	4.0 (61.5)	80.0 (64.2)	28.9 (38.1)	15.5 (46.2)	44.4 (41.2)	96.7 (44.9)	17.0 (55.1)	113.7 (46.7)	19.1 (49.1)	4.6 (60.0)	23.7 (51.6)
L.S.D. (0.05)	0.33	7.36	1.23	0.91	1.49	3.79	0.94	3.80	0.77	0.28	0.94
L.S.D. (0.01)	0.45	10.0	1.67	1.24	2.02	5.15	1.28	5.17	1.04	0.38	1.28

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over uninoculated control

Table 8: Effect of different initial inoculum levels of *Meloidogyne incognita* on root-knot disease development and nematode multiplication on tomato cv. K-25 under pot conditions^a

Initial Inoculum Levels (Pi = J ₂ /5 kg soil)	Final Nematode Population (Pf)			Reproduction Factor (Rf=Pf/Pi)	^b Root-knot Index (RKI)
	Root (Total)	Soil (5 kg soil)	Total		
0	-	-	-	-	-
2500	16350	22000	38350	15.3	1.50
5000	25542	32000	57542	11.5	2.25
10000	37380	46000	83380	8.3	2.95
15000	45825	56000	101825	6.8	3.75
20000	51800	64000	115800	5.8	4.00
25000	55420	68000	123420	4.9	4.00
L.S.D. (0.05)	1083.39	1347.91	2672.37	0.49	0.12
L.S.D. (0.01)	1474.06	1842.59	3640.84	0.67	0.17

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

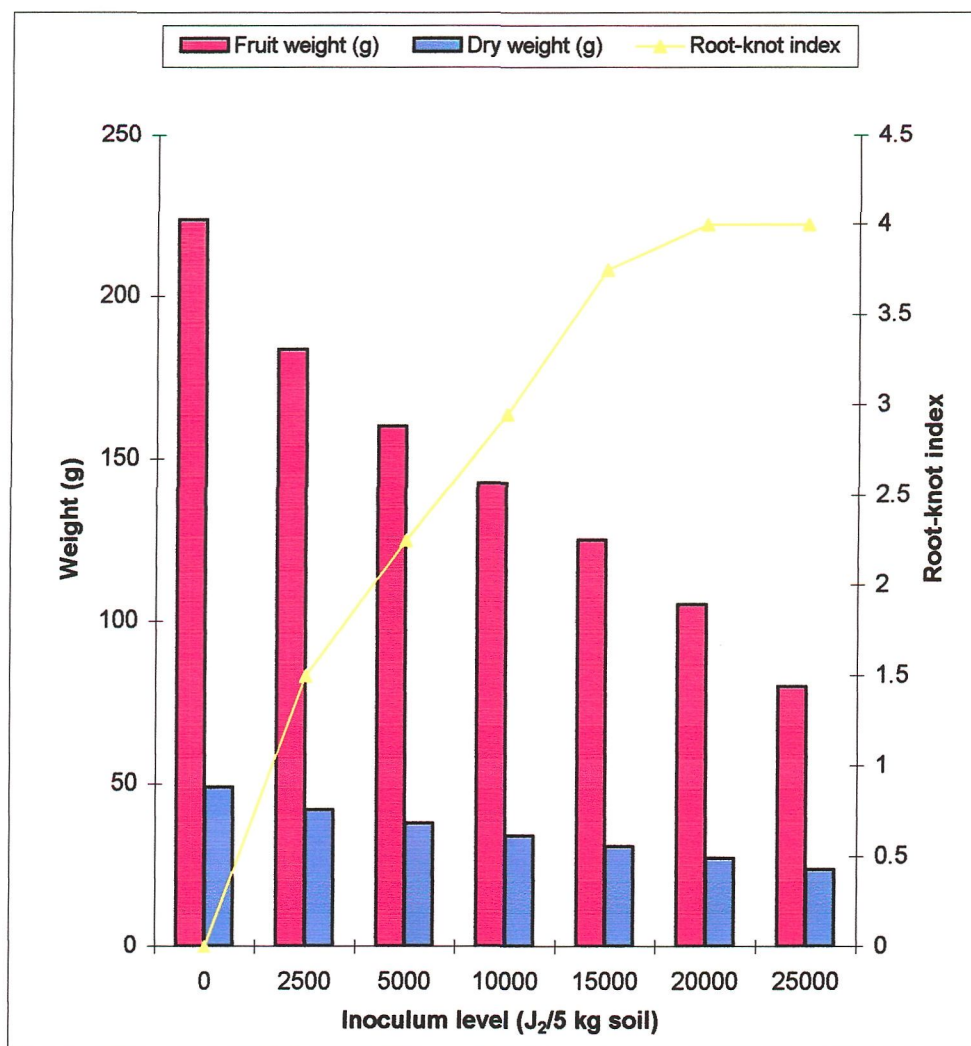


Fig. 3: Effect of different initial inoculum levels of *M. incognita* on root-knot disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

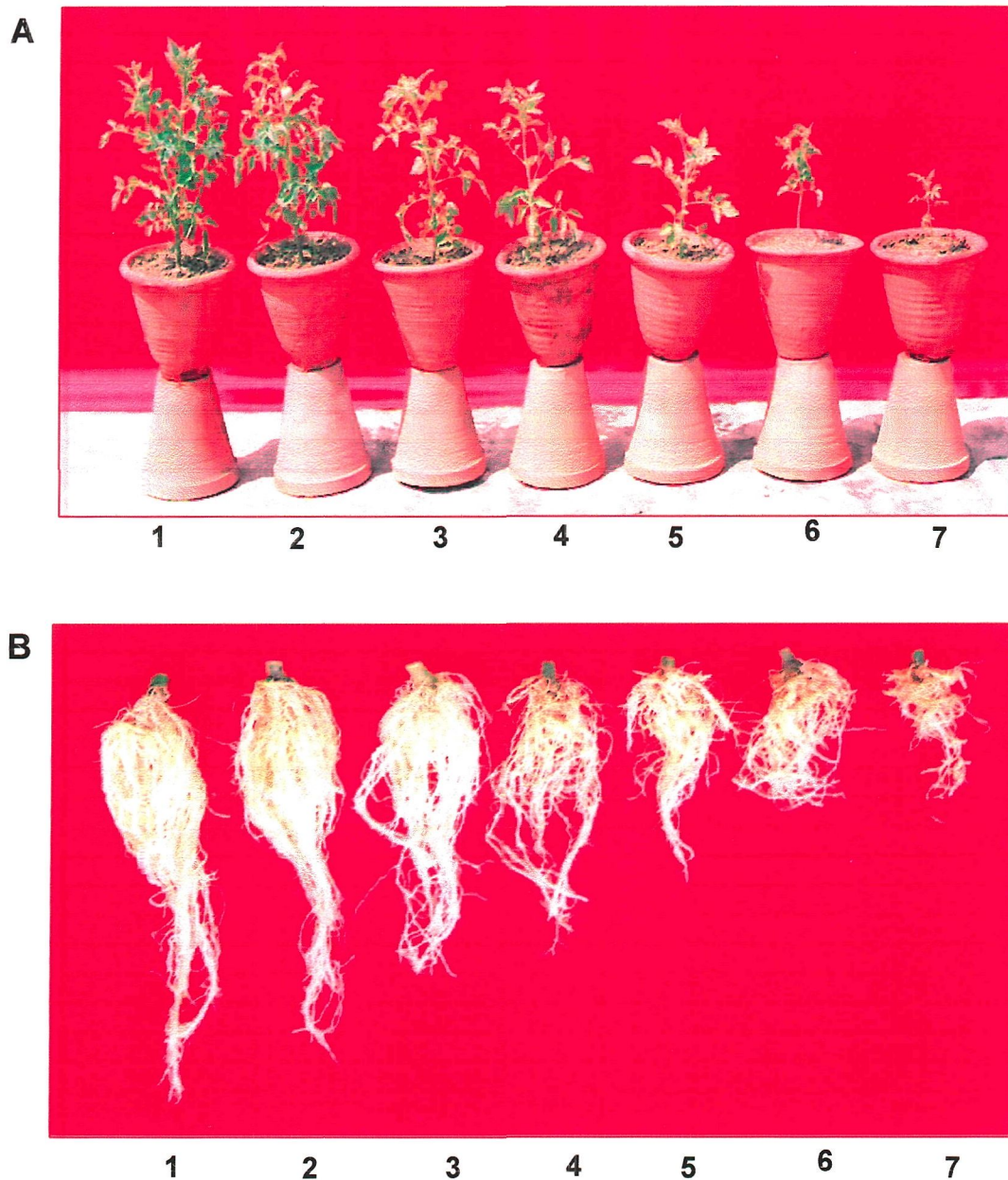


Plate 5: Effect of different inoculum levels of *M. incognita* on the aerial growth (A) and root-knot development on tomato cv. K-25 (B)

1= Uninoculated control	5= 15,000 J_2 /5 kg soil
2= 2500 J_2 /5 kg soil	6= 20,000 J_2 /5 kg soil
3= 5000 J_2 /5 kg soil	7= 25,000 J_2 /5 kg soil
4= 10,000 J_2 /5 kg soil	

Table 9: Effect of different initial inoculum levels of *Fusarium oxysporum* on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions^a

Initial Inoculum Levels (cfu/5 kg soil)	Number of Fruits	Fruit Weight (g)	Plant Length (cm)			Plant Fresh Weight (g)			Plant Dry Weight (g)			^b Percent Root Infection
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total	
0.0	10.6	225.0	47.0	28.7	75.7	176.5	37.7	214.2	38.0	11.5	49.5	-
2.5x10 ⁶	10.2 (3.8) ^c	215.0 (4.4)	45.5 (3.2)	28.1 (2.1)	73.6 (2.8)	169.9 (3.7)	36.5 (3.2)	206.4 (3.6)	36.4 (4.2)	11.1 (3.5)	47.5 (4.0)	4.5
5.0x10 ⁶	9.8 (7.5)	203.5 (9.6)	43.3 (7.9)	27.2 (5.2)	70.5 (6.9)	161.1 (8.7)	35.1 (6.9)	196.2 (8.4)	34.2 (10.0)	10.5 (8.7)	44.7 (9.7)	9.5
7.5x10 ⁶	9.0 (15.1)	185.0 (17.8)	40.1 (14.7)	25.5 (11.1)	65.6 (13.3)	147.0 (16.7)	32.4 (14.0)	179.7 (16.1)	31.1 (18.2)	9.7 (15.6)	40.8 (17.6)	17.5
10 ⁷	8.0 (24.5)	160.0 (28.9)	36.5 (22.3)	23.3 (18.8)	59.8 (21.0)	134.5 (23.8)	29.9 (20.7)	164.4 (23.2)	27.9 (26.6)	8.7 (24.3)	36.6 (26.1)	30.0
1.25x10 ⁷	7.2 (32.1)	135.0 (40.0)	33.0 (29.8)	20.7 (27.9)	53.7 (29.1)	123.3 (30.1)	27.1 (28.1)	150.4 (29.7)	25.5 (32.9)	7.9 (31.3)	33.4 (32.5)	42.5
1.5x10 ⁷	6.4 (39.6)	112.5 (50.0)	30.7 (34.7)	18.3 (36.2)	49.0 (35.3)	111.0 (37.1)	24.3 (35.5)	135.3 (36.8)	22.7 (40.3)	7.1 (38.3)	29.8 (39.8)	50.5
L.S.D. (0.05)	0.23	8.68	1.71	0.92	2.29	6.94	1.33	8.34	1.43	0.37	1.68	1.45
L.S.D. (0.01)	0.31	11.75	2.33	1.25	3.11	9.44	1.81	11.34	1.94	0.50	2.28	1.97

^aEach value is an average of five replicates

^bPercent root infection by *F. oxysporum*

^cFigures in parentheses are percent reduction over uninoculated control

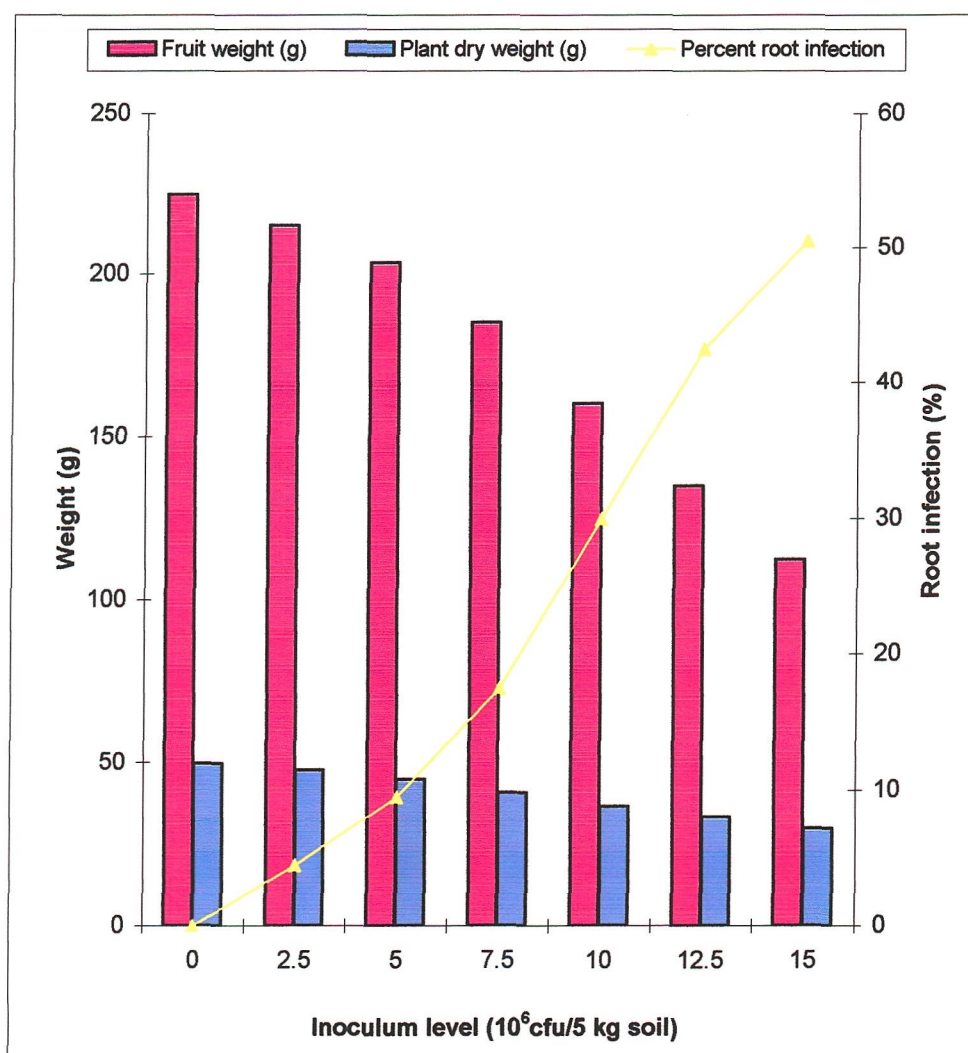


Fig. 4: Effect of different initial inoculum levels of *F. oxysporum* on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

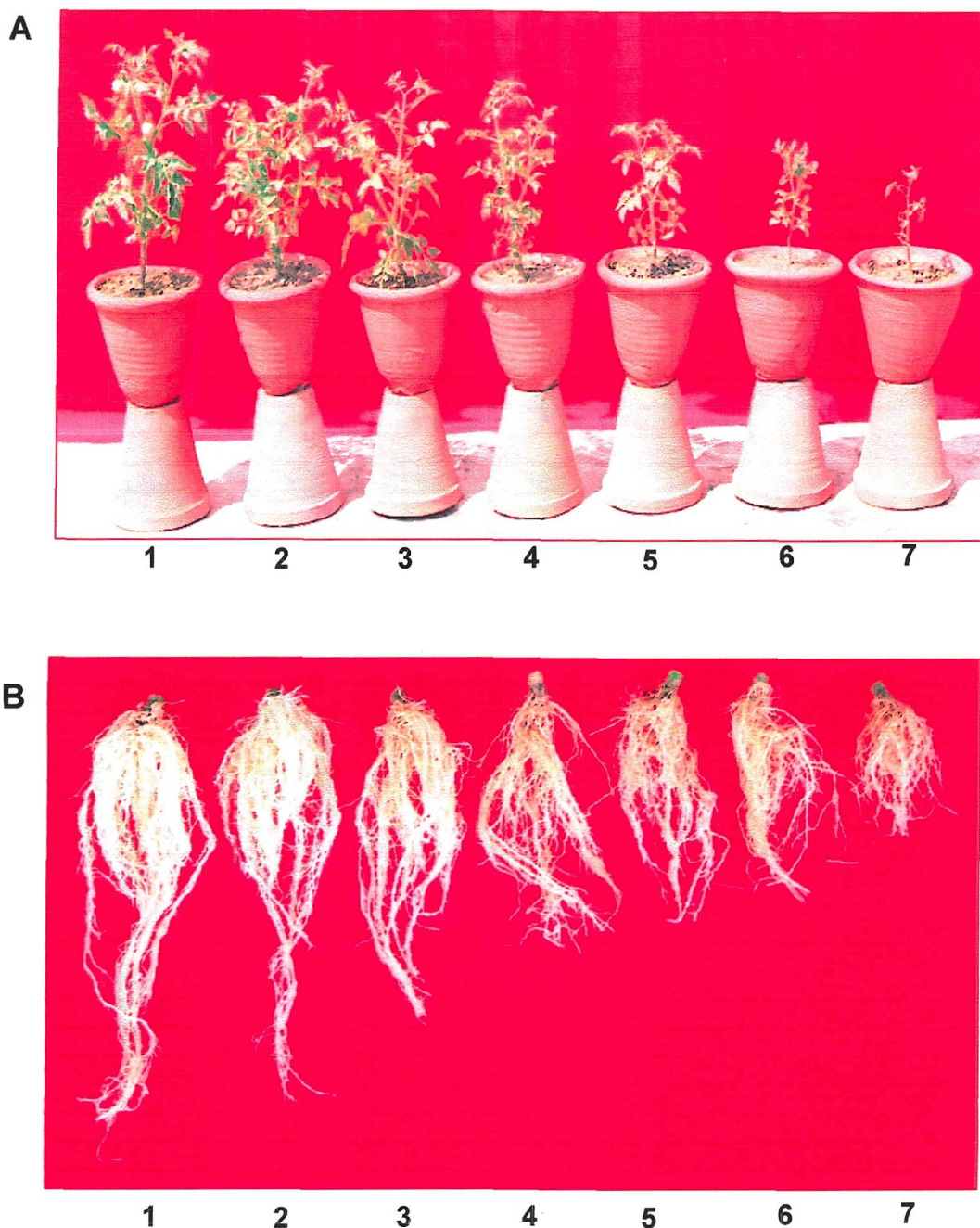


Plate 6: Effect of different initial inoculum levels of *F. oxysporum* on the aerial growth (A) and symptoms development on roots of tomato cv. K-25 (B)

1= Uninoculated control
 2= 2.5×10^6 cfu/5 kg soil
 3= 5.0×10^6 cfu/5 kg soil
 4= 7.5×10^6 cfu/5 kg soil

5= 10^7 cfu/5 kg soil
 6= 1.25×10^7 cfu/5 kg soil
 7= 1.5×10^7 cfu/5 kg soil

2.2 *F. oxysporum* alone

Studies regarding the effect of different Pi of *F. oxysporum* on the disease development, plant growth and fruit yield of tomato cv. K-25 indicated that the significant reduction ($P \leq 0.05$) in number of fruits, fruit yield, shoot-root fresh and dry weights was obtained at 5.0×10^6 cfu/5 kg soil (Table 9; Fig. 4; Plate 6). Fruit yield and plant dry weight was significantly ($P \leq 0.05$) reduced at lowest Pi (2.5×10^6 cfu/5 kg soil). The highest reduction in number of fruits, fruit yield, shoot-root fresh and dry weights was 39.6, 50.0, 37.1, 35.5, 40.3 and 38.3%, respectively at highest Pi of 1.5×10^7 cfu/5 kg soil as compared to uninoculated control. Extent of reduction in various test parameters was increased with the corresponding increase in Pi and differences in all the test parameters between corresponding Pi were significant ($P \leq 0.05$). However, plant length and fresh weight were found to be nonsignificant at lowest Pi as compared to uninoculated control.

Infection in roots was observed between 4.5% to 50.5% at Pi 2.5×10^6 to 1.5×10^7 cfu/5 kg (Table 9; Fig. 4). Differences in the extent of PRI between all the corresponding Pi were highly significant ($P \leq 0.05$).

2.3 *R. solani* alone

Data regarding the effect of different Pi of *R. solani* on the disease development, plant growth and fruit yield of tomato cv. K-25 showed that there was a corresponding decrease in number of fruits, fruit yield, shoot-root fresh and dry weights (Table 10; Fig. 5; Plate 7). The highest reduction in number of fruits, fruit yield, shoot-root fresh and dry weights was 53.8, 60.8, 42.1, 41.4, 45.6 and 45.5%, respectively was recorded at highest Pi of 15.0 g mycelium/5 kg soil as compared to uninoculated control. Analyses of data indicated that significant ($P \leq 0.05$) reduction in shoot-root fresh and dry weights, and fruit yield was found at Pi of 2.5 g mycelium/5 kg soil. Extent of reduction in various test parameters increased with the corresponding increase in Pi and the differences in test parameters between corresponding Pi were significant ($P \leq 0.05$).

The percent root infection due to *R. solani* increased with the increasing Pi. At the lowest Pi (2.5 g mycelium/5 kg soil), infection was 5.0% and at the highest Pi (15.0 g

Table 10: Effect of different initial inoculum levels of *Rhizoctonia solani* on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions^a

Initial inoculum levels (g mycelium/5 kg soil)	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			^b Percent root infection
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total	
0.0	10.4	223.0	46.0	27.5	73.5	176.0	37.0	213.0	37.3	11.0	48.3	-
2.5	9.8 (5.8) ^c	206.5 (7.4)	44.2 (3.9)	26.7 (2.9)	70.9 (3.5)	168.1 (4.5)	35.7 (3.5)	203.8 (4.3)	35.5 (4.8)	10.6 (3.6)	46.1 (4.6)	5.0
5.0	9.4 (9.6)	195.0 (12.6)	41.3 (10.2)	25.1 (8.7)	66.4 (9.7)	156.0 (11.4)	33.5 (9.5)	189.5 (11.0)	32.5 (12.9)	9.9 (10.0)	42.4 (12.2)	11.5
7.5	8.4 (19.2)	170.0 (23.8)	37.1 (19.3)	23.2 (15.6)	60.3 (18.0)	142.0 (19.3)	30.5 (17.6)	172.5 (19.0)	29.3 (21.4)	9.0 (18.2)	38.3 (20.7)	25.0
10.0	7.2 (30.8)	143.5 (35.7)	32.7 (28.9)	21.1 (23.3)	53.8 (26.8)	126.7 (28.4)	27.7 (25.1)	154.4 (27.5)	25.7 (31.1)	8.0 (27.3)	33.7 (30.2)	40.0
12.5	5.8 (44.2)	107.5 (51.8)	29.0 (37.0)	18.8 (31.6)	47.8 (35.0)	114.1 (35.2)	24.3 (34.3)	138.4 (35.0)	23.1 (38.1)	6.9 (37.3)	30.0 (37.9)	52.5
15.0	4.8 (53.8)	87.5 (60.8)	25.2 (45.2)	16.5 (40.0)	41.7 (43.3)	101.9 (42.1)	21.7 (41.4)	123.6 (42.0)	20.3 (45.6)	6.0 (45.5)	26.3 (45.5)	65.0
L.S.D. (0.05)	0.30	7.84	1.27	0.73	1.16	6.97	1.46	7.93	1.42	0.43	1.95	2.06
L.S.D. (0.01)	0.40	10.66	1.73	0.99	1.57	9.48	1.95	10.78	1.93	0.58	2.65	2.80

^aEach value is an average of five replicates

^bPercent root infection by *R. solani*

^cFigures in parentheses are percent reduction over uninoculated control

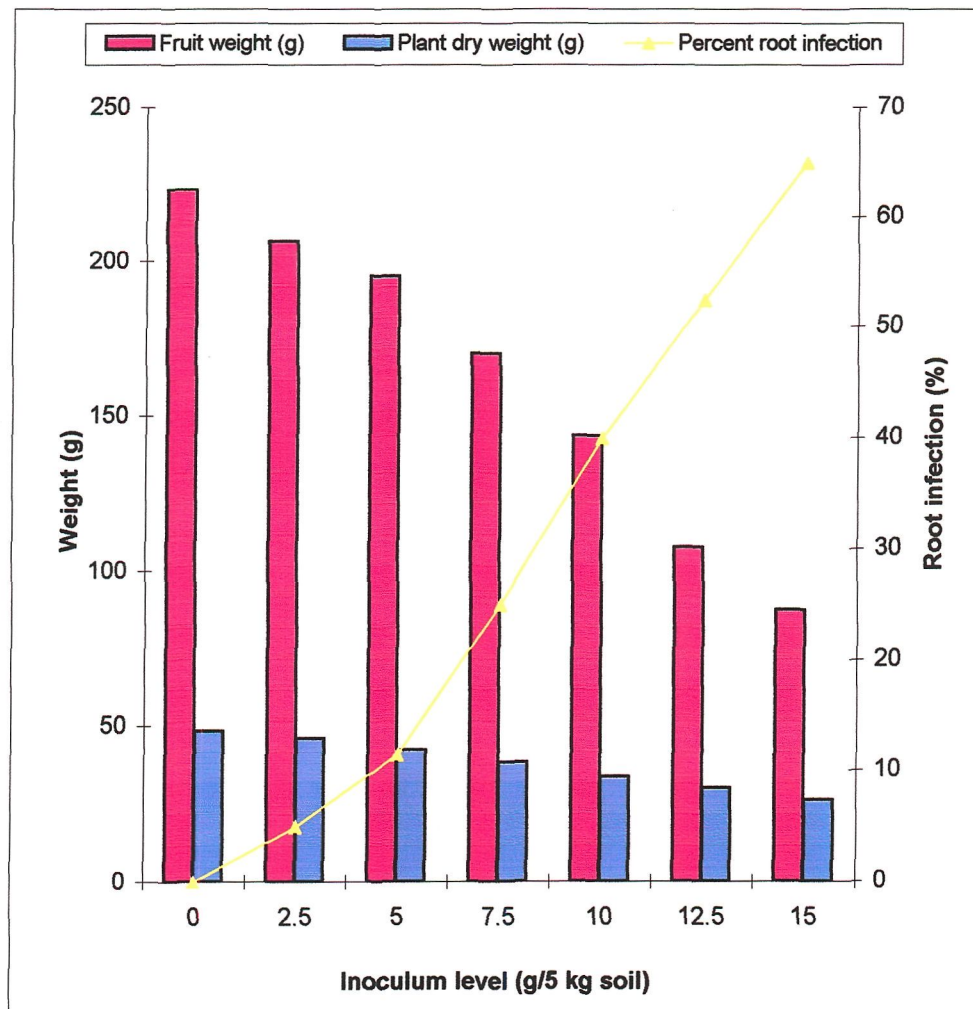


Fig. 5: Effect of different initial inoculum levels of *R. solani* on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

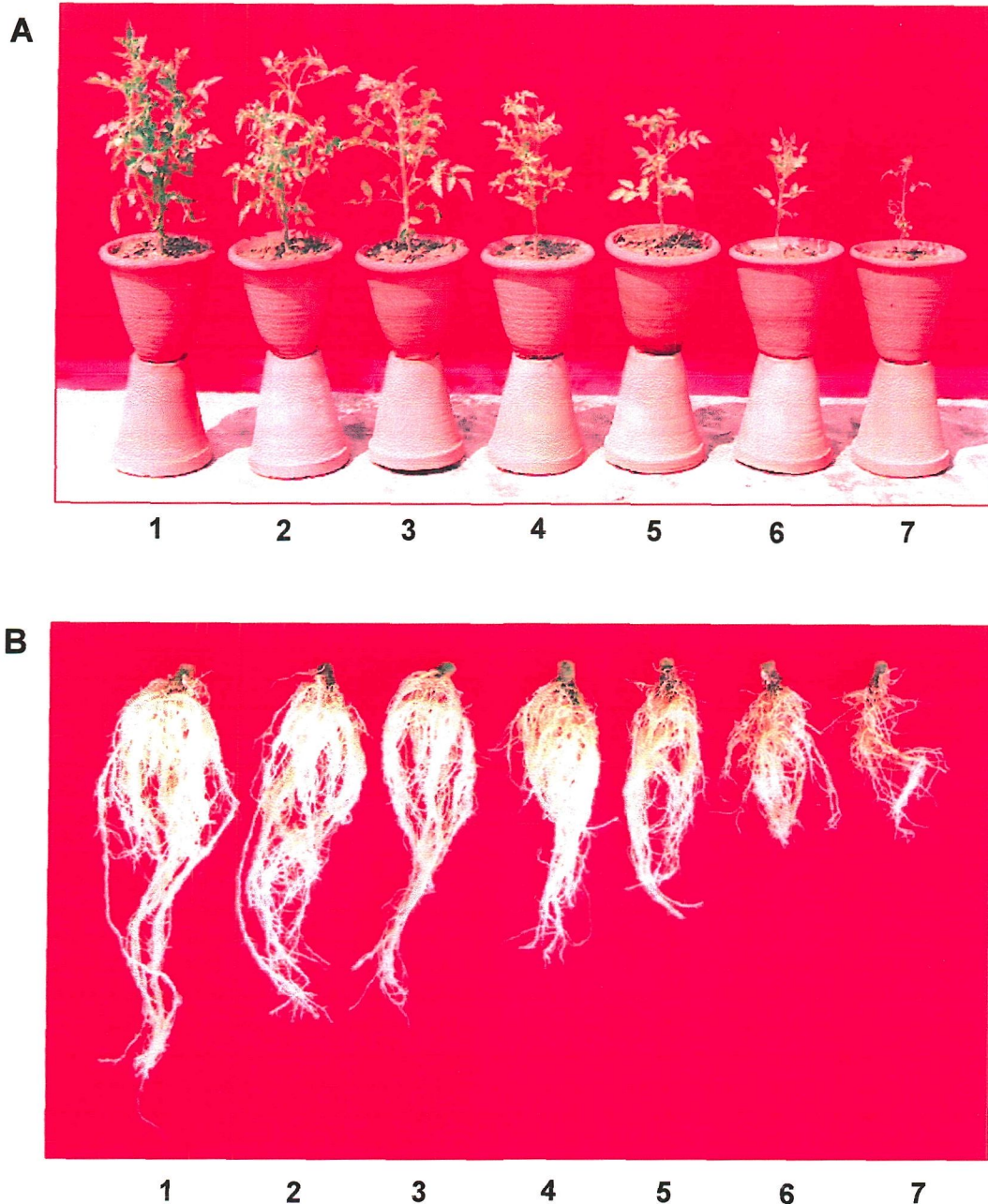


Plate 7: Effect of different initial inoculum levels of *R. solani* on the aerial growth (A) and symptoms development on roots of tomato cv. K-25 (B)

1= Uninoculated control
 2= 2.5 g mycelium/5 kg soil
 3= 5.0 g mycelium/5 kg soil
 4= 7.5 g mycelium/5 kg soil

5= 10.0 g mycelium/5 kg soil
 6= 12.5 g mycelium/5 kg soil
 7= 15.0 g mycelium/5 kg soil

mycelium/5 kg soil) it was 65%. Significant ($P \leq 0.05$) differences were observed in the extent of root infection among all the corresponding Pi (Table 10; Fig. 5).

3. Interactive effect of *M. incognita*, *F. oxysporum* and *R. solani*, alone and in combination on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

Data regarding the interactive effect of *M. incognita*, *F. oxysporum* and *R. solani* on the disease development, plant growth and fruit yield of tomato cv. K-25 indicated that in general, *M. incognita* was found significantly ($P \leq 0.05$) more pathogenic in reducing the number of fruits, fruit yield, shoot-root fresh and dry weights of plants than *R. solani* and *F. oxysporum* alone [Table 11; Fig. 6; Plate 8 (i), (ii)]. Highest suppression of test parameters was found in plants inoculated simultaneously with *M. incognita*, *F. oxysporum* and *R. solani* followed by *M. incognita* seven days prior to *F. oxysporum* and *R. solani*, *F. oxysporum* and *R. solani* seven days prior to *M. incognita*, *M. incognita* and *R. solani* simultaneously, *M. incognita* seven days prior to *R. solani*, *M. incognita* and *F. oxysporum* simultaneously, *M. incognita* seven days prior to *F. oxysporum*, *F. oxysporum* and *R. solani* simultaneously, *R. solani* seven days prior to *M. incognita* and *F. oxysporum* seven days prior to *M. incognita*, respectively.

All the treatments significantly ($P \leq 0.05$) reduced fruit yield and plant growth parameters as compared to uninoculated control. Differences in suppressive effects of various treatments on fruit yield and dry weight of tomato were significant ($P \leq 0.05$). However, few nonsignificant differences were also noticed. Highest reduction in fruit yield (83.3%), shoot dry weight (78.0%) and root dry weight (81.3%) was found in plants inoculated with *M. incognita*, *F. oxysporum* and *R. solani* simultaneously and lowest reduction in fruit yield (19.8%), shoot dry weight (18.4%) and root dry weight (15.9%) respectively was obtained in plants inoculated with *F. oxysporum* alone.

In general, root-knot development and reproduction factor of *M. incognita* were decreased in presence of fungal pathogens, while extent of root infection by the fungal pathogens increased in presence of nematodes (Table 12; Fig. 7). Highest Rf (11.8) and RKI (2.90) were observed in plants inoculated with nematode alone followed by *M. incognita* prior to *R. solani* (8.0 and 2.60), *M. incognita* prior to *F. oxysporum* (7.5 and 2.50), *M. incognita* and *R. solani* simultaneously (6.8 and 2.45), *M. incognita* and *F.*

Table 11: Effect of *Meloidogyne incognita* (5000 J₂/5 kg soil), *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) alone and in combinations on plant growth and fruit yield of tomato cv. K-25^a under pot conditions

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)	
			Shoot height	Root length	Shoot	Root	Shoot	Root
Uninoculated Control	10.6	224.5	45.5	27.5	73.0	36.7	210.2	10.7
<i>M. incognita</i> alone	8.0 (24.5) ^b	165.0 (26.5)	36.9 (18.9)	22.0 (20.0)	138.0 (20.5)	28.9 (21.3)	166.9 (20.6)	8.0 (25.2)
<i>F. oxysporum</i> alone	8.8 (17.0)	180.0 (19.8)	38.5 (15.4)	24.3 (11.6)	143.3 (17.4)	31.5 (14.2)	174.8 (16.8)	9.0 (15.9)
<i>R. solani</i> alone	8.4 (20.8)	173.5 (22.7)	37.0 (18.7)	23.3 (15.3)	139.5 (19.6)	30.3 (17.4)	169.8 (19.2)	8.7 (18.9)
<i>F. oxysporum</i> + <i>R. solani</i>	6.0 (43.4)	120.0 (46.5)	29.7 (34.7)	17.5 (36.5)	104.0 (40.1)	20.9 (43.1)	124.9 (40.6)	5.9 (44.9)
<i>M. incognita</i> + <i>F. oxysporum</i>	5.4 (49.1)	112.0 (50.1)	27.7 (39.1)	15.7 (42.9)	95.5 (45.0)	18.5 (49.6)	114.0 (45.8)	5.1 (52.3)
<i>M. incognita</i> + <i>R. solani</i>	4.8 (54.7)	96.5 (57.0)	25.2 (44.6)	14.5 (47.3)	86.9 (50.1)	16.5 (55.0)	103.4 (50.8)	4.5 (57.9)
<i>M. incognita</i> + <i>F. o. + R. s.</i>	2.4 (77.4)	37.5 (83.3)	15.5 (65.9)	6.0 (78.2)	41.7 (76.0)	7.7 (79.0)	49.4 (76.5)	2.0 (81.3)
^c <i>M. i.</i> (pre) + <i>F. o.</i> (post)	5.6 (47.2)	115.0 (48.8)	29.3 (35.6)	17.1 (37.8)	97.0 (44.1)	18.9 (48.5)	115.9 (44.9)	5.3 (50.5)
<i>M. i.</i> (pre) + <i>R. s.</i> (post)	5.2 (50.9)	103.5 (53.9)	27.0 (40.7)	15.9 (42.2)	92.7 (46.7)	17.5 (52.3)	110.2 (47.6)	4.9 (54.2)
<i>M. i.</i> (pre) + <i>F. o. & R. s.</i> (post)	3.0 (71.7)	51.5 (77.1)	18.0 (60.4)	6.7 (75.6)	46.0 (73.5)	8.7 (76.3)	54.7 (74.0)	2.3 (78.5)
^d <i>F. o.</i> (pre) + <i>M. i.</i> (post)	6.4 (39.6)	132.5 (41.0)	33.5 (26.4)	18.3 (33.5)	111.0 (36.0)	22.1 (39.8)	133.1 (36.7)	6.3 (41.1)
<i>R. s.</i> (pre) + <i>M. i.</i> (post)	6.2 (41.5)	127.5 (43.2)	33.1 (27.3)	18.0 (34.5)	108.1 (37.7)	21.7 (40.9)	129.8 (38.2)	6.0 (43.9)
<i>F. o. & R. s.</i> (pre) + <i>M. i.</i> (post)	3.6 (66.0)	67.5 (69.9)	20.7 (54.5)	9.0 (67.3)	62.5 (64.0)	12.3 (66.5)	74.8 (64.4)	3.3 (69.2)
L.S.D. 0.05	0.28	8.98	1.88	1.06	7.13	1.44	8.84	0.24
L.S.D. 0.01	0.37	12.21	2.58	1.44	9.70	1.98	12.02	0.32
								2.48

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over uninoculated control

^c*M. incognita* inoculated seven days prior to fungal pathogen

^dFungal pathogens inoculated seven days prior to *M. incognita*.

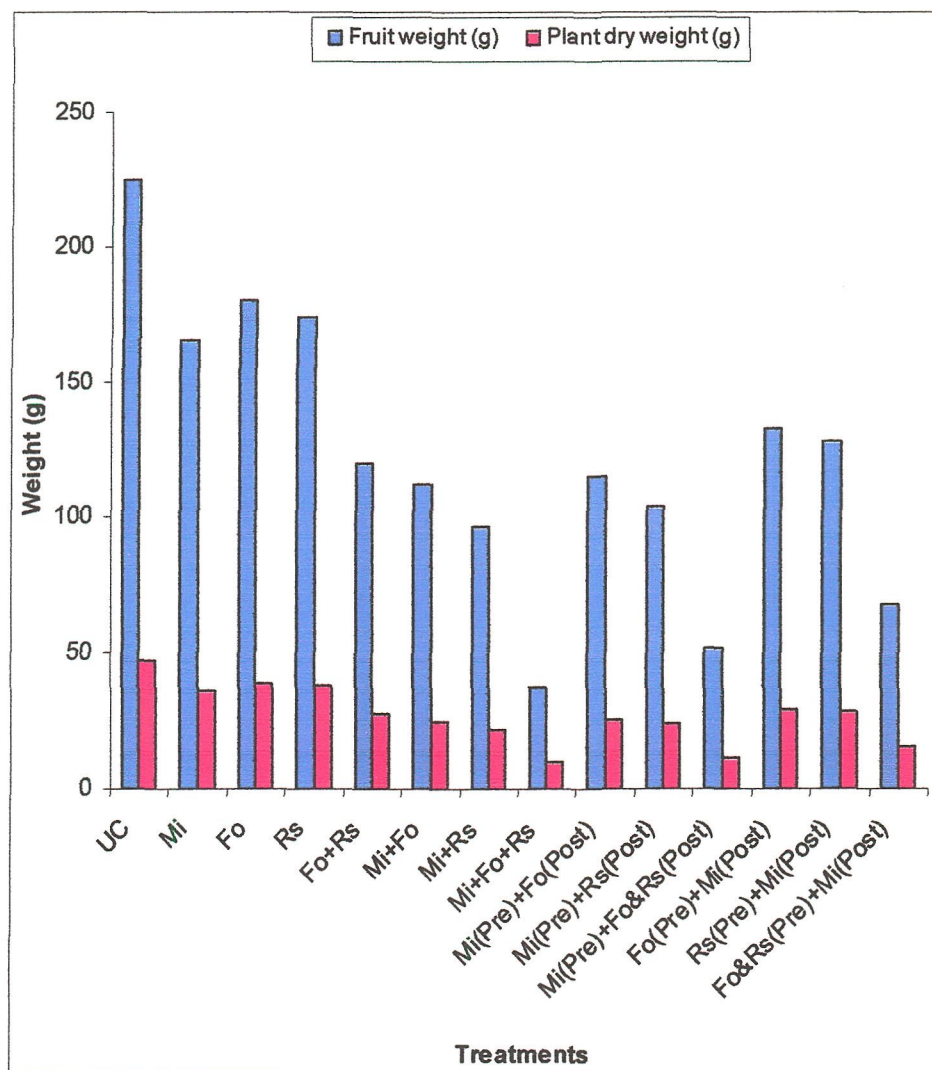


Fig. 6: Effect of *M. incognita* (5000 J2/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) on plant growth and fruit yield of tomato cv. K-25 under pot conditions

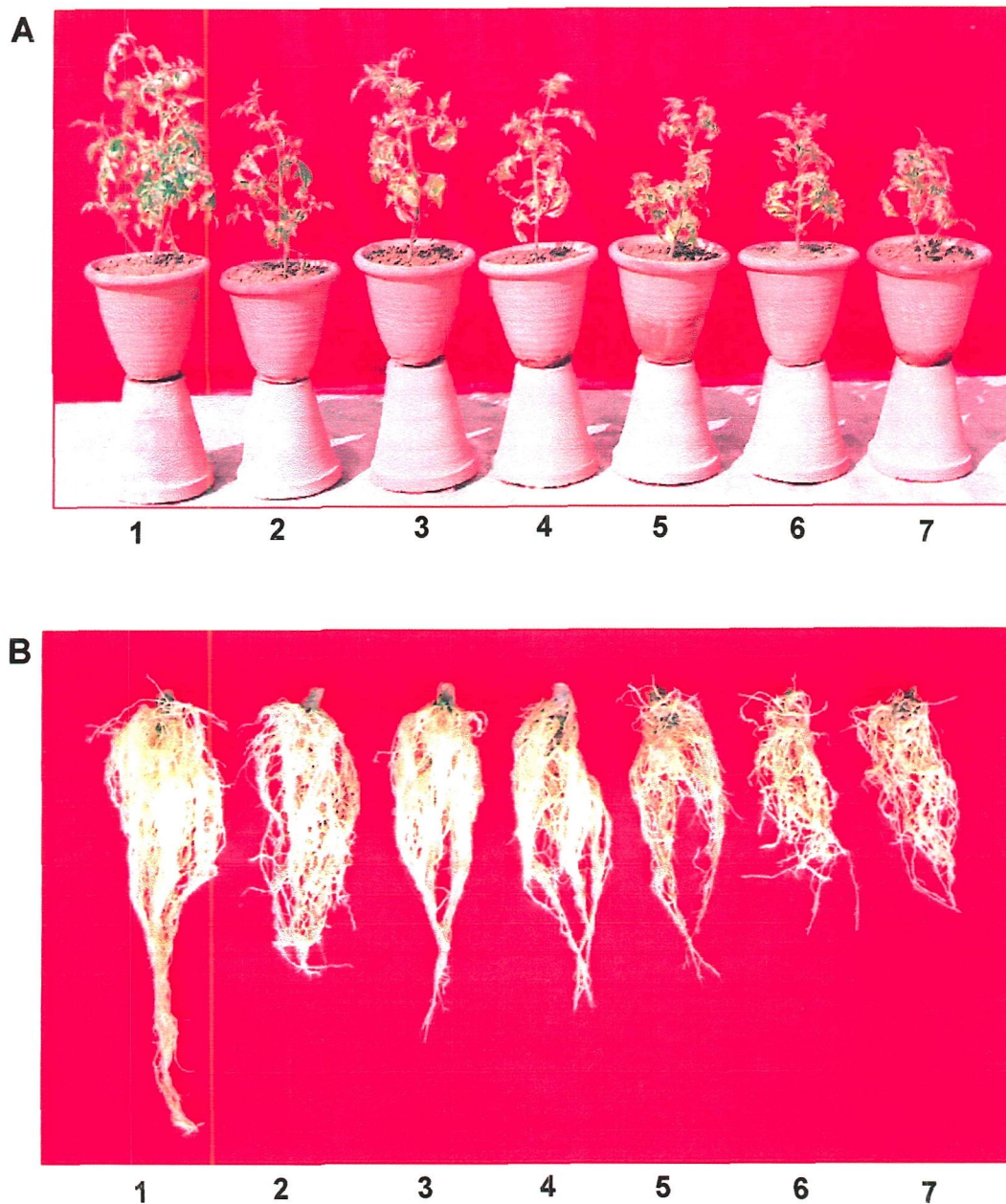


Plate 8 (i): Interactive effects of *M. incognita*, *F. oxysporum* and *R. solani* on aerial growth (A) and symptoms development on roots of tomato cv. K-25 (B)

1= Uninoculated control
 2= 5000 J₂ of *M. i.*/5 kg soil
 3= 7.5×10^6 cfu of *F. o.*/5 kg soil
 4= 7.5 g mycelium of *R. s.*/5 kg soil

5= *F. o.* + *R. s.*/5 kg soil
 6= *M. i.* + *F. o.*/5 kg soil
 7= *M. i.* + *R. s.*/5 kg soil



Plate 8 (ii): Interactive effects of *M. incognita*, *F. oxysporum* and *R. solani* on aerial growth (A) and symptoms development on roots of tomato cv. K-25 (B).

1= Uninoculated control

8= *M. i.* + *F. o.* + *R. s.*/5 kg soil

9= *M. i.* (pre)+*F. o.* (post)/5 kg soil

10= *M. i.* (pre)+*R. s.* (post)/5 kg soil

11= *M. i.* (pre)+*F. o.* & *R. s.* (post)/5 kg soil

12= *F. o.* (pre)+*M. i.* (post)/5 kg soil

13= *R. s.* (pre)+*M. i.* (post)/5 kg soil

14= *F. o.* + *R. s.* (pre)+*M. i.* (post)/5 kg soil

Table 12. Effect of *Meloidogyne incognita* (5000 J2/5 kg soil), *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) alone and in combinations on nematode multiplication, root-knot disease development and percent root infection by fungal pathogens on tomato cv. K-25 under pot conditions^a

Treatments	Final nematode population			Reproduction factor	^b Root-knot index	^c Percent root infection
	Root (Total)	Soil (5kg)	Total			
Uninoculated Control	-	-	-	-	-	-
<i>M. incognita</i> alone	24852	34000	58852	11.8	2.9	-
<i>F. oxysporum</i> alone	-	-	-	-	-	20.0
<i>R. solani</i> alone	-	-	-	-	-	27.0
<i>F. oxysporum</i> + <i>R. solani</i>	-	-	-	-	-	50.0
<i>M. incognita</i> + <i>F. oxysporum</i>	11100	20000	31100	6.2	2.4	47.5
<i>M. incognita</i> + <i>R. solani</i>	12210	22000	34210	6.8	2.5	57.5
<i>M. incognita</i> + <i>F. o.</i> + <i>R. s.</i>	3080	14000	17080	3.4	1.5	80.0
^c <i>M. i.</i> (pre) + <i>F. o.</i> (post)	13608	24000	37608	7.5	2.5	45.0
<i>M. i.</i> (pre) + <i>R. s.</i> (post)	14000	26000	40000	8.0	2.6	55.0
<i>M. i.</i> (pre) + <i>F. o.</i> & <i>R. s.</i> (post)	4176	16000	20176	4.0	1.8	72.5
^d <i>F. o.</i> (pre) + <i>M. i.</i> (post)	10608	16000	26608	5.3	2.0	39.0
<i>R. s.</i> (pre) + <i>M. i.</i> (post)	12586	18000	30586	6.1	2.3	43.5
<i>F. o.</i> & <i>R. s.</i> (pre) + <i>M. i.</i> (post)	2952	10000	12952	2.6	1.0	66.7
L.S.D. 0.05	545.86	1089.63	1418.89	0.4	0.1	3.2
L.S.D. 0.01	743.78	1491.59	1933.36	0.5	0.2	4.3

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

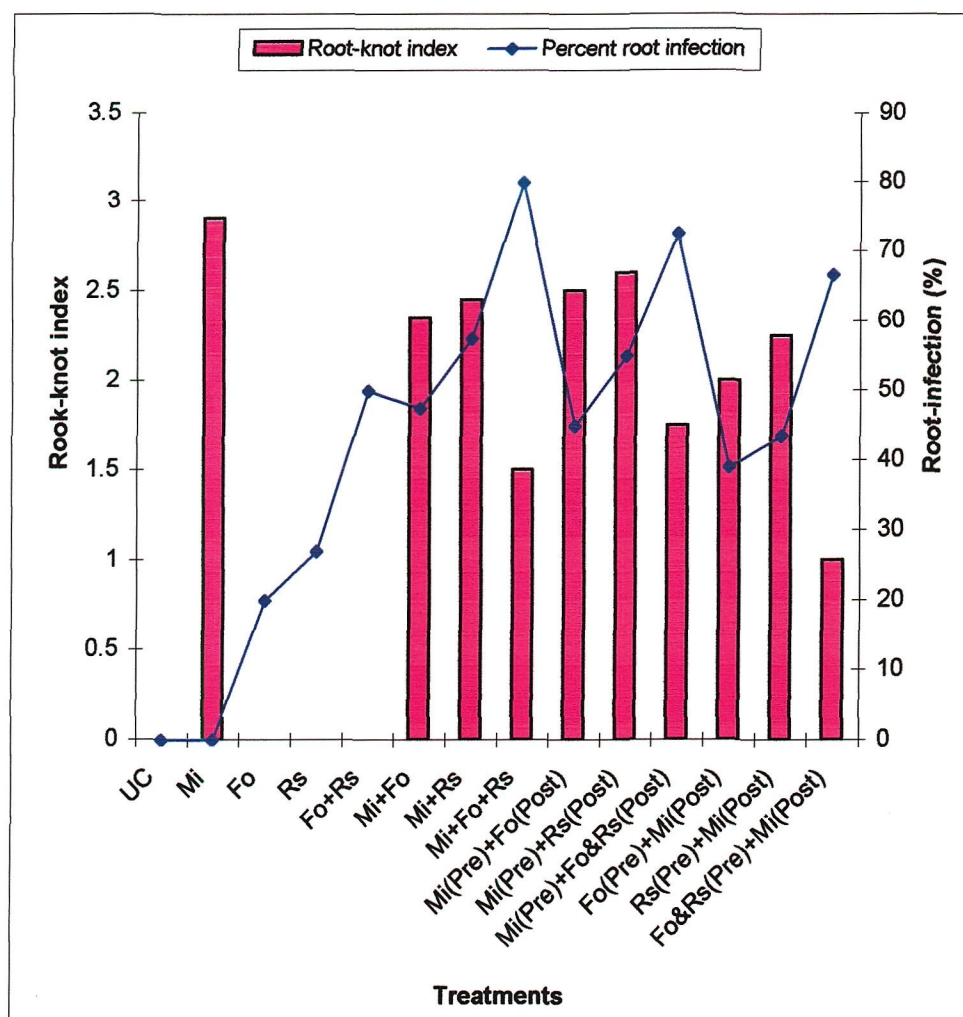


Fig.7: Effect of *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) on root-knot disease development and percent root infection by fungal pathogens on tomato cv. K-25 under pot conditions

UC = Untreated control
 Mi = *Meloidogyne incognita*
 Fo = *Fusarium oxysporum*
 Rs = *Rhizoctonia solani*

oxysporum simultaneously (6.2 and 2.35), *R. solani* prior to *M. incognita* (6.1 and 2.25), *F. oxysporum* prior to *M. incognita* (5.3 and 2.00), *M. incognita* prior to *F. oxysporum* and *R. solani* (4.0 and 1.75), *M. incognita*, *F. oxysporum* and *R. solani* simultaneously (3.4 and 1.50) and *F. oxysporum* and *R. solani* prior to *M. incognita* (2.6 and 1.00), respectively.

The highest PRI (80.0%) by fungal pathogens was observed in plants inoculated with *M. incognita*, *F. oxysporum* and *R. solani* simultaneously followed by *M. incognita* prior to *F. oxysporum* and *R. solani* (72.5%), *F. oxysporum* and *R. solani* prior to *M. incognita* (66.7%), *M. incognita* and *R. solani* simultaneously (57.5%), *M. incognita* prior to *R. solani* (55.0%), *F. oxysporum* and *R. solani* simultaneously (50.0%), *M. incognita* and *F. oxysporum* simultaneously (47.5%), *M. incognita* prior to *F. oxysporum* (45.0%), *R. solani* prior to *M. incognita* (43.5%) and *F. oxysporum* prior to *M. incognita* (39.0%), respectively. Differences in RKI were mostly significant ($P \leq 0.05$) among various treatments with few nonsignificant differences were also observed. Similarly, differences in PRI by fungal pathogens were mostly significant ($P \leq 0.05$) among various treatments.

4. In-vitro, evaluation of biocontrol agents, organic amendments and pesticides for their efficacy against *F. oxysporum* and *R. solani* alone

4.1 Screening of isolates of *T. harzianum* and *T. virens* against *F. oxysporum* and *R. solani*

Results (Table 13) indicated that all the isolates of *Trichoderma* significantly ($P \leq 0.01$) inhibited the growth of *F. oxysporum*. Among the isolates of *T. harzianum*, isolate TH-AG-2 was the best antagonist in inhibiting the growth of *F. oxysporum* (90.9%) followed by isolates TH-UIP-2, TH-JP-2, TH-H-3, TH-SP-1, TH-MN-2, TH-M-7, TH-AL, TH-K-9, TH-AL, TH-AG-5 and TH-SJ, respectively [Plate 9 (i) A]. Analyses of data indicated that the differences among all the isolates were found mostly significant. However, few nonsignificant ($P \leq 0.05$) differences between TH-MN-2 and TH-SP-1; TH-M-7, TH-K-9 and TH-BS-6; and TH-AG-5 and TH-SJ were also observed.

Among the isolates of *T. virens*, isolate TV-K-3 was highly inhibitory to the growth of *F. oxysporum* (86.4%) followed by TV-AL-1, TV-M-5, TV-AG-3, TV-H and

Table 13: *In vitro* screening of isolates of *Trichoderma harzianum* and *T. virens* against *Fusarium oxysporum* and *Rhizoctonia solani*

Isolates of <i>Trichoderma</i> species	<i>Fusarium oxysporum</i>		Radial growth in diameter (cm)	
		Percent reduction	<i>Rhizoctonia solani</i>	Percent reduction
<i>Trichoderma harzianum</i>				
TH-AG-2	0.8	90.9	3.8	57.8
TH-M-7	1.5	83.0	4.4	51.1
TH-AG-5	1.7	80.7	4.1	54.4
TH-MN-2	1.4	84.1	3.2	64.4
TH-UIP-2	0.9	89.8	4.5	50.0
TH-JP-2	1.0	88.6	4.4	51.1
TH-SJ	1.7	80.7	2.9	67.8
TH-AL	1.6	81.8	3.9	56.7
TH-K-9	1.5	83.0	2.6	71.1
TH-BS-6	1.5	83.0	4.5	50.0
TH-H-3	1.3	85.2	0.0	100.0
TH-SP-1	1.4	84.1	0.9	90.0
<i>T. virens</i>				
TV-K-3	1.2	86.4	0.7	92.2
TV-H	1.7	80.7	2.5	72.2
TV-M-1	2.9	67.0	4.1	54.4
TV-AG-3	1.6	81.8	4.3	52.2
TV-M-5	1.5	83.0	4.5	50.0
TV-AL-1	1.3	85.2	3.9	56.7
Control	8.8	-	9.0	-
L.S.D. (0.05)	0.07		0.13	
L.S.D. (0.01)	0.09		0.17	

^aEach value is an average of five replicates

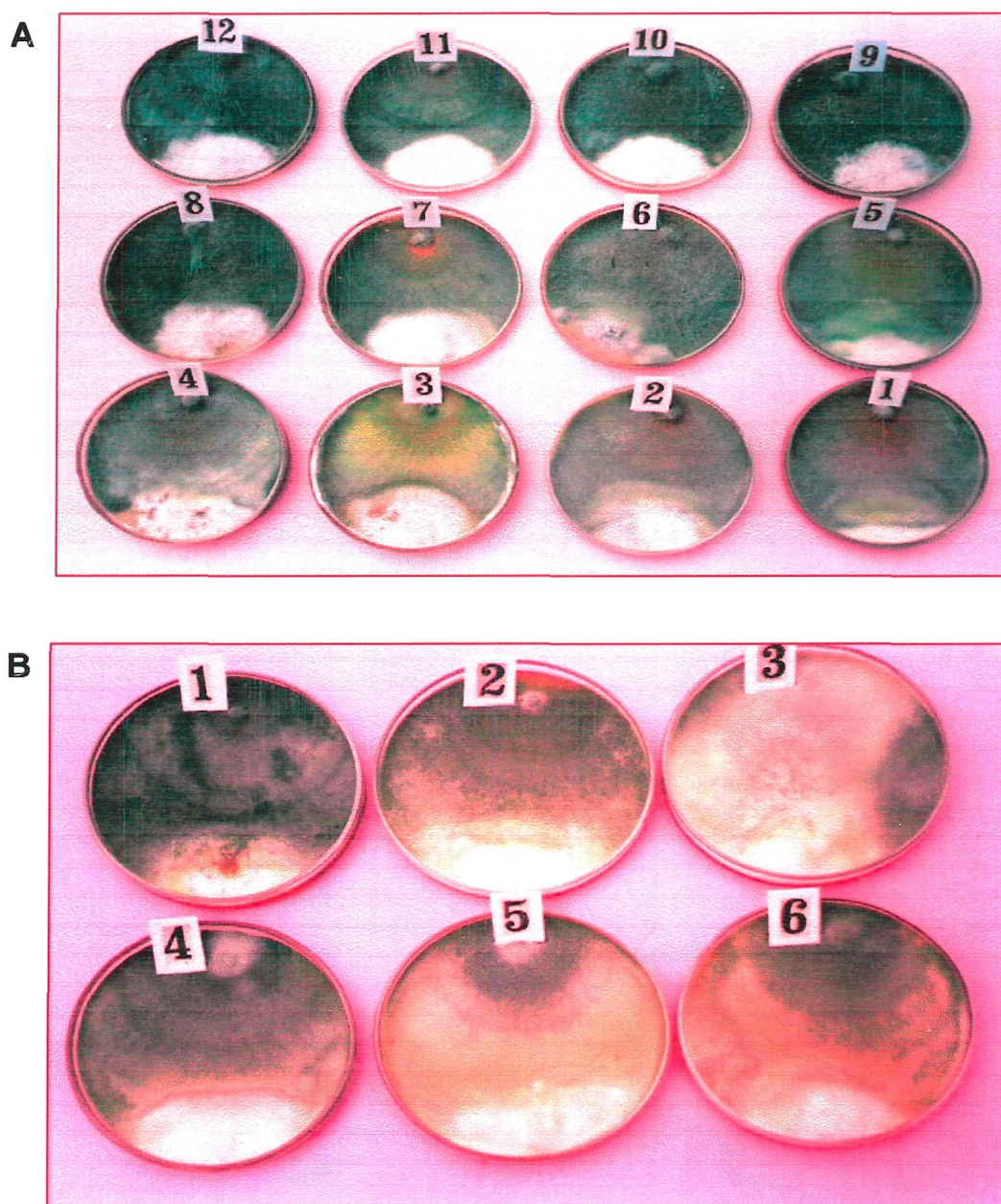


Plate 9 (i): Petriplates showing antagonistic effect of different isolates of *T. harzianum* and *T. virens* against *F. oxysporum* in dual culture technique

- | | | |
|----------------|-------------|---------------|
| (A) 1= TH-AG-2 | 7= TH-SJ | (B) 1= TV-K-3 |
| 2= TH-M-7 | 8= TH-AL | 2= TV-H |
| 3= TH-AG-5 | 9= TH-K-9 | 3= TV-M-1 |
| 4= TH-MN-2 | 10= TH-BS-6 | 4= TV-AG-3 |
| 5= TH-UIP-2 | 11= TH-H-3 | 5= TV-M-5 |
| 6= TH-JP-2 | 12= TH-SP-1 | 6= TV-AL-1 |

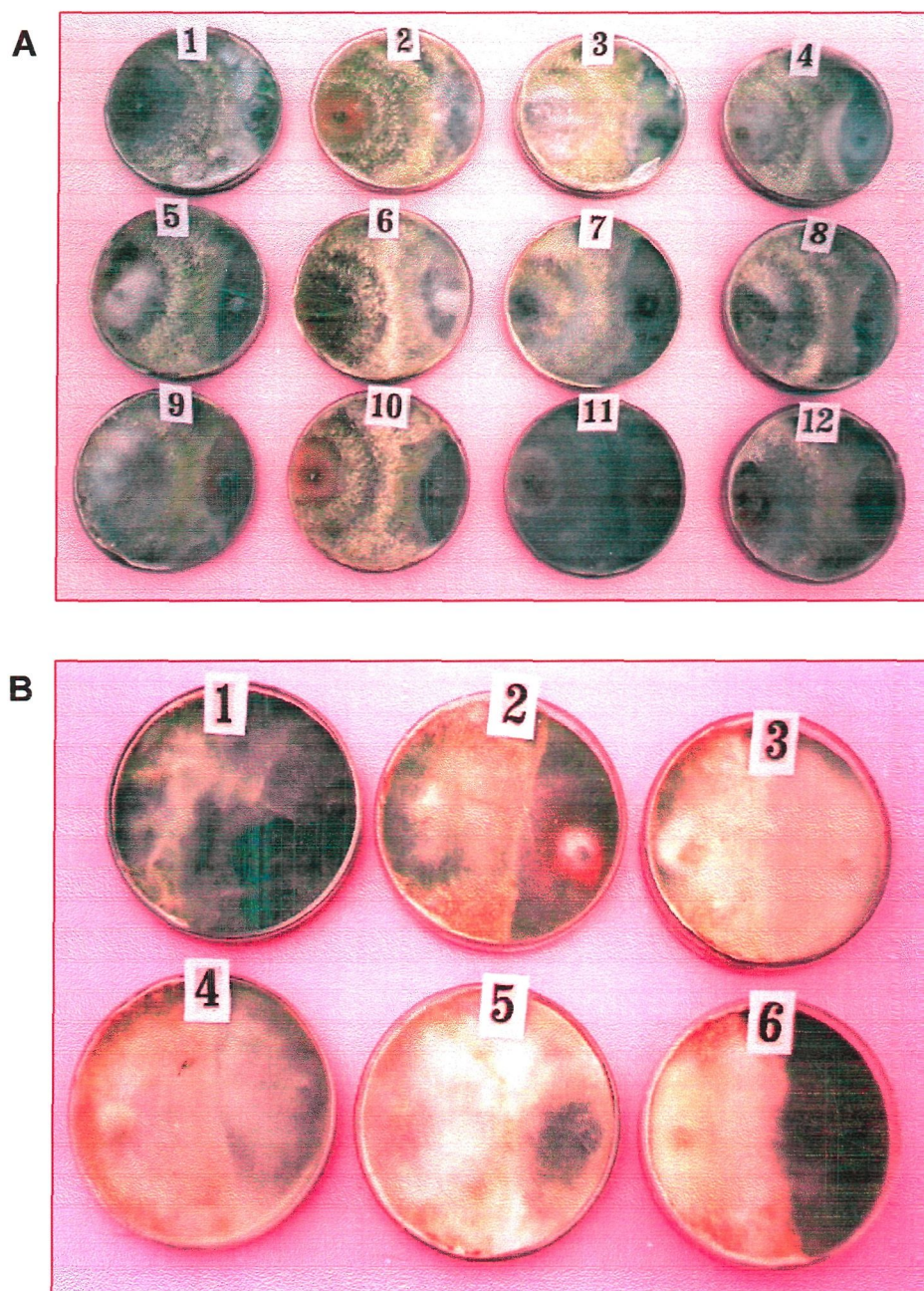


Plate 9 (ii): Petriplates showing antagonistic effect of different isolates of *T. harzianum* and *T. virens* against *R. solani* in dual culture technique

- | | | |
|----------------|-------------|---------------|
| (A) 1= TH-AG-2 | 7= TH-SJ | (B) 1= TV-K-3 |
| 2= TH-M-7 | 8= TH-AL | 2= TV-H |
| 3= TH-AG-5 | 9= TH-K-9 | 3= TV-M-1 |
| 4= TH-MN-2 | 10= TH-BS-6 | 4= TV-AG-3 |
| 5= TH-UIP-2 | 11= TH-H-3 | 5= TV-M-5 |
| 6= TH-JP-2 | 12= TH-SP-1 | 6= TV-AL-1 |

TV-M, respectively [Plate 9 (i) B]. Analyses of data indicated that the differences among all the isolates were significant ($P \leq 0.05$).

Similarly, all the isolates of *T. harzianum* were found effective ($P \leq 0.01$) in suppressing the growth of *R. solani*. Among *T. harzianum* isolates, isolate TH-H-3 was highly antagonistic to *R. solani* followed by isolates TH-SP-1, TH-K-9, TH-SJ, TH-MN-2, TH-AG-2, TH-AL, TH-AG-5, TH-M-7, TH-JP-2, TH-UIP-2 and TH-BS-6, respectively [Plate 9 (ii) A]. Differences among all the isolates in suppressing the growth of *R. solani* were found mostly significant ($P \leq 0.05$). Among the *T. virens* isolates, highest growth inhibition of *R. solani* was found with isolate TV-K-3 followed by TV-H, TV-AL-1, TV-M-1, TV-AG-3 and TV-M-5, respectively [Plate 9 (ii) B]. Differences among all the isolates were highly significant ($P \leq 0.05$).

Data indicated that *T. harzianum* isolate TH-H-3 was found the best in overall performance as it inhibited the 85% and 100% growth of *F. oxysporum* and *R. solani*, respectively. Similarly, *T. virens* isolate TV-K-3 was highly antagonistic to both the pathogenic fungi as it inhibited the 86.4% and 92.2% growth of *F. oxysporum* and *R. solani*, respectively. Therefore, these two biocontrol agents were selected for further studies.

4.2 Screening of isolates of *P. fluorescens* against *F. oxysporum* and *R. solani*

Data (Table 14) regarding the screening of isolates of *P. fluorescens* against *F. oxysporum* and *R. solani* showed that in general, all the isolates were significantly inhibited the growth of *F. oxysporum* as compared to control. Isolate PS-4 was highly antagonistic (65.5%) to *F. oxysporum* followed by NK-2, H-2, PS-5, NK-1, K-3, M-3, PS-9, K-1, BW-2, M-5, UIP-2, AG-1, BW-1, RGP-1, UIP-1 and AG-4, respectively [Plate 10 (i)]. Analyses of the data indicated that differences among isolates of *P. fluorescens* in inhibition of the growth of *F. oxysporum* were mostly significant ($P \leq 0.05$) with few nonsignificant differences among the isolates were also observed.

Similarly, most of the isolates inhibited the growth of *R. solani* except the isolates RGP-1 and BW-1. Isolate PS-4 was the best antagonist followed by PS-5, PS-9, AG-1, NK-1, UIP-2, NK-2, K-3, BW-2, H-2, UIP-1, K-1, M-3, M-5, AG-4, RGP-1 and BW-1, respectively [Plate 10 (ii)]. Differences in inhibition on the growth of *R. solani* by

Table 14: *In vitro* screening of *Pseudomonas fluorescens* isolates against *Fusarium oxysporum* and *Rhizoctonia solani*

Isolates of <i>Pseudomonas fluorescens</i>	Radial growth in diameter (cm)		
	<i>Fusarium oxysporum</i>	Percent reduction	<i>Rhizoctonia solani</i>
AG-1	6.0	31.0	3.7
AG-4	6.9	20.7	8.0
RGP-1	6.8	21.8	9.0
BW-1	6.3	27.6	9.0
BW-2	4.6	47.1	4.1
PS-4	3.0	65.5	2.4
PS-5	3.9	55.2	2.6
PS-9	4.3	51.7	3.0
UIP-1	6.5	25.3	4.2
UIP-2	5.5	36.8	4.0
NK-1	3.9	55.2	3.9
NK-2	3.4	60.9	4.0
H-2	3.7	57.5	4.1
K-1	4.5	48.3	4.2
K-3	3.9	55.2	4.0
M-3	4.2	51.7	4.2
M-5	5.1	41.4	4.3
Control	8.7	-	9.0
L.S.D. (0.05)	0.16		0.19
L.S.D. (0.01)	0.22		0.26

^aEach value is an average of five replicates



Plate 10 (i): Petriplates showing antagonistic effect of different isolates of *P. fluorescens* against *F. oxysporum* in dual culture technique

(A) C= control	5= BW-2	(B) 1= UIP-1	6= K-1
1= AG-1	6= PS-4	2= UIP-2	7= K-2
2= AG-4	7= PS-7	3= NK-1	8= M-3
3= RGP-1	8= PS-9	4= NK-2	9= M-5
4= BW-1		5= H-2	

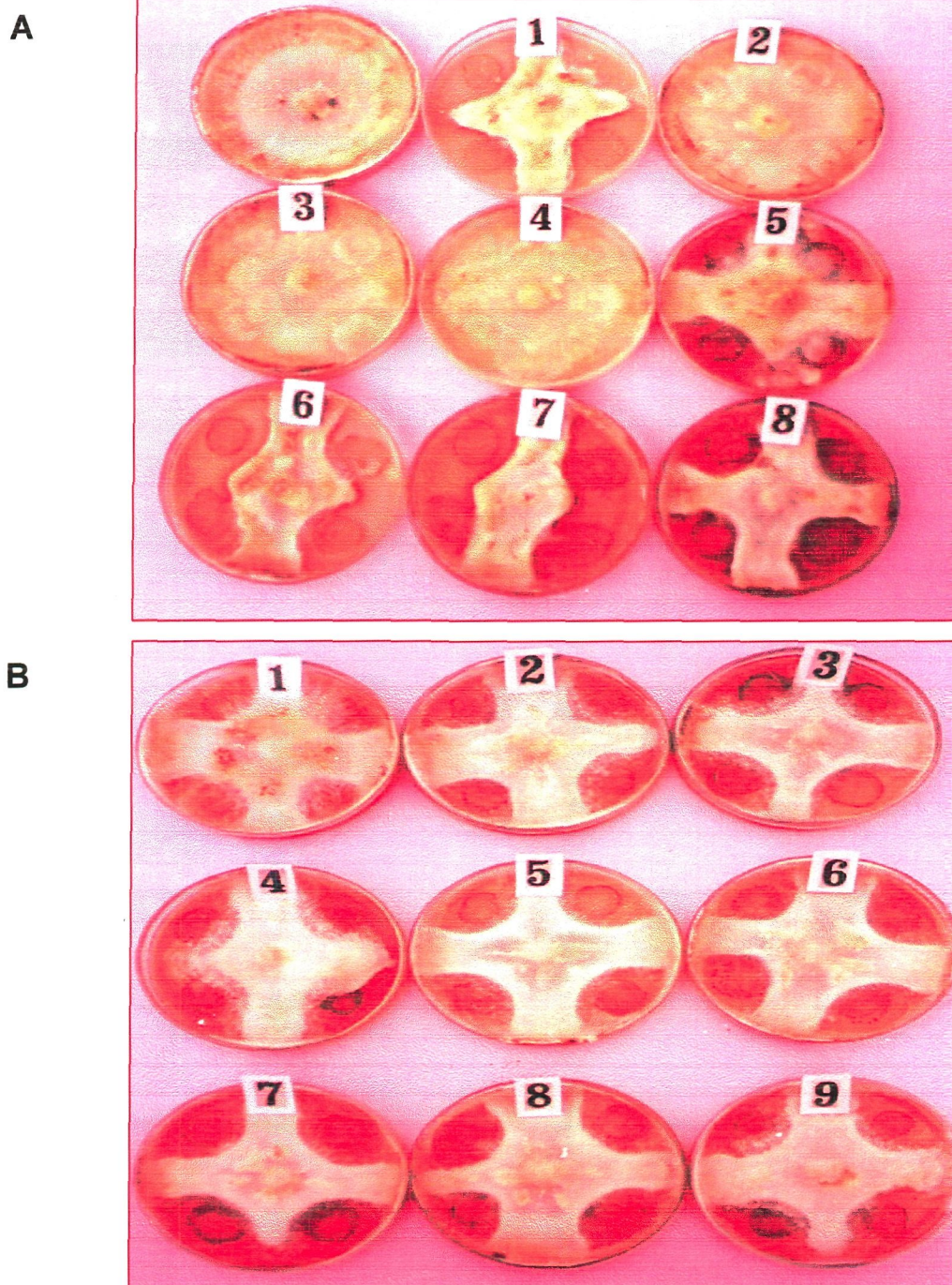


Plate 10 (ii): Petriplates showing antagonistic effect of different isolates of *P. fluorescens* against *R. solani* in dual culture technique

(A) C= control	5= BW-2	(B) 1= UIP-1	6= K-1
1= AG-1	6= PS-4	2= UIP-2	7= K-3
2= AG-4	7= PS-7	3= NK-1	8= M-3
3= RGP-1	8= PS-9	4= NK-2	9= M-5
4= BW-1		5= H-2	

Table 15: Comparative efficacy of organic additives and pesticides against *Fusarium oxysporum* and *Rhizoctonia solani* in vitro

Treatments	Radial growth in diameter (cm)			
	<i>Fusarium oxysporum</i>	Percent reduction	<i>Rhizoctonia solani</i>	Percent reduction
Farm yard manure	8.4	4.5	9.0	0.0
Neem seed powder	7.0	20.5	8.5	5.6
Carbofuran	7.7	12.5	8.8	2.2
Topsin-M	0.0	100.0	0.0	100.0
Bavistin	0.0	100.0	0.0	100.0
Control	8.8	-	9.0	-
L.S.D. (0.05)	0.13		0.14	
L.S.D. (0.01)	0.18		0.18	

^aEach value is an average of five replicates

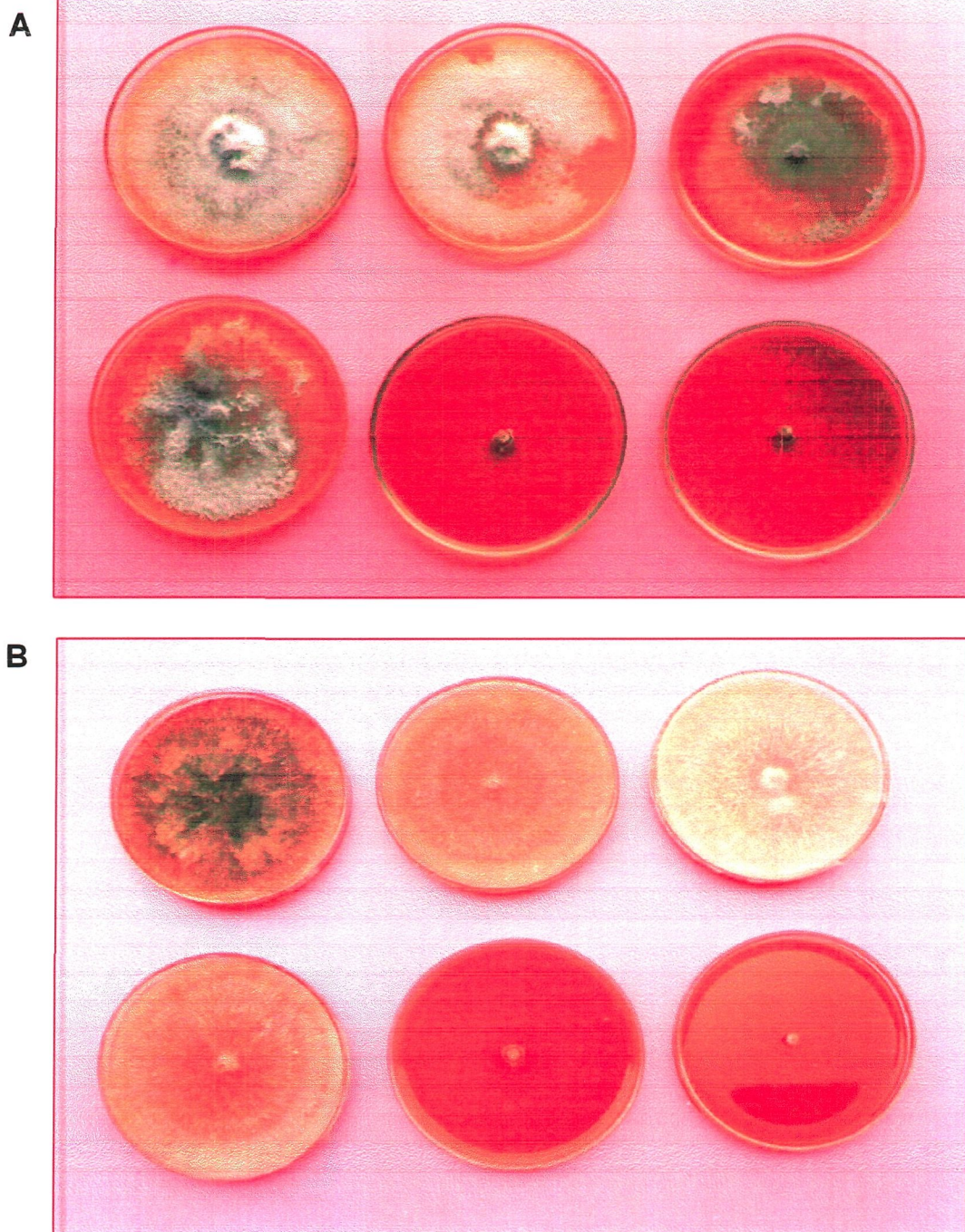


Plate 11: Petriplates showing inhibitory effect of organic additives and pesticides against *F. oxysporum* (A) and *R. solani* (B)

1= Untreated control
2= Farmyard manure
3= Neem seed powder

4= Carbofuran
5= Topsin-M
6= Bavistin

different isolates was mostly significant ($P \leq 0.05$) with few nonsignificant differences between isolates were also observed.

4.3 Efficacy of organic amendments and pesticides against *F. oxysporum* and *R. solani*

Data regarding the comparative efficacy of organic additives and pesticides against *F. oxysporum* and *R. solani* *in vitro* showed that bavistin and topsin-M were the best treatments in decreasing (100%, 100%) the growth of both the test pathogens followed by neem seed powder (21.1%, 13.3%), carbofuran (11.1%, 3.3%) and farmyard manure (6.7%, 1.1%), respectively (Table 15; Plate 11 A, B).

Analyses of data indicated that differences in reduction in the growth of *F. oxysporum* plates treated with different additives were found to be mostly significant ($P \leq 0.01$), except a nonsignificant difference between bavistin and topsin-M. Also the differences in reduction in the growth of *R. solani* plates treated with different additives were mostly significant ($P \leq 0.01$). However, nonsignificant differences between bavistin and topsin-M, and carbofuran and farmyard manure were also observed.

5. Comparative efficacy of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* alone and in combinations, on tomato cv. K-25 under pot conditions

5.1 *M. incognita* alone

Data (Table 16) regarding the comparative efficacy of different additives against *M. incognita* indicated that the application of various treatments significantly ($P \leq 0.05$) increased the plant growth and fruit yield of tomato as compared to untreated inoculated plants [Fig. 8; Plate 12 (i), (ii)].

Analyses of data indicated that differences in plant dry weight and fruit yield of tomato plants treated with different additives were mostly significant ($P \leq 0.05$). However, highest plant dry weight (46.0 g) and fruit yield (212.0 g) were obtained in plants treated with carbofuran followed by neem seed powder, *T. harzianum*, *P. fluorescens*, *T. virens*, bavistin, topsin-M and farm yard manure, respectively.

Table 16: Comparative efficacy of biocontrol agents, organic additives and pesticides on the plant growth and fruit yield of tomato cv. K-25 inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total
Untreated-uninoculated Control	10.6	224.5	45.0	28.0	73.0	174.5	38.0	212.5	37.9	11.7	49.6
Untreated inoculated control	8.0 (24.5) ^b	162.5 (27.6)	37.7 (16.2)	22.5 (19.6)	60.2 (17.5)	138.7 (20.5)	29.7 (21.8)	168.4 (20.8)	29.5 (22.2)	8.9 (23.9)	38.4 (22.6)
<i>T. harzianum</i>	10.0 (5.7)	207.5 (7.6)	42.8 (4.9)	25.7 (8.7)	68.5 (6.2)	162.0 (7.2)	34.9 (8.2)	196.9 (7.3)	34.7 (8.4)	10.6 (10.3)	45.3 (8.7)
<i>T. virens</i>	9.6 (9.4)	198.5 (11.6)	41.4 (8.0)	25.0 (10.7)	66.4 (9.0)	154.3 (11.6)	33.3 (12.4)	187.6 (11.7)	32.9 (13.2)	10.0 (14.5)	42.9 (13.5)
<i>P. fluorescens</i>	9.8 (7.5)	202.5 (9.8)	42.0 (6.7)	25.3 (9.6)	67.3 (7.8)	157.7 (9.6)	33.9 (10.8)	191.6 (9.8)	33.7 (11.1)	10.2 (12.8)	43.9 (11.5)
Farm yard manure	8.6 (18.9)	175.0 (22.0)	41.0 (8.9)	24.0 (16.0)	65.0 (11.0)	148.0 (15.2)	31.8 (16.3)	179.8 (15.4)	31.6 (16.6)	9.5 (18.8)	41.1 (17.1)
Neem Seed Powder	10.0 (5.7)	209.0 (6.9)	43.2 (4.0)	26.0 (7.1)	69.2 (5.2)	163.5 (6.3)	35.2 (7.4)	198.7 (6.5)	34.9 (7.9)	10.6 (9.4)	45.5 (8.3)
Carbofuran	10.2 (3.8)	212.0 (5.6)	44.0 (2.2)	26.7 (4.6)	70.7 (3.2)	165.0 (5.4)	35.5 (6.6)	200.5 (5.6)	35.3 (6.9)	10.7 (8.5)	46.0 (7.3)
Topsin-M	9.0 (15.1)	185.0 (17.6)	41.1 (8.7)	24.3 (13.2)	65.4 (10.4)	149.3 (14.4)	32.1 (15.5)	181.4 (14.6)	31.9 (15.8)	9.6 (17.9)	41.5 (16.3)
Bavistin	9.2 (13.2)	189.5 (15.6)	41.2 (8.4)	24.5 (12.5)	65.7 (10.0)	151.5 (13.2)	32.6 (14.2)	184.1 (13.4)	32.3 (14.8)	9.8 (16.2)	42.1 (15.1)
LSD (0.05)	0.33	8.62	1.62	1.10	2.77	5.46	1.72	7.42	1.65	0.46	1.42
LSD (0.01)	0.44	11.72	2.20	1.49	3.77	7.43	2.34	10.9	2.24	0.63	1.93

^aEach value is an average of five replicates.

^bFigures in parentheses are percent reduction over untreated uninoculated control

Table 17: Comparative efficacy of different biocontrol agents, organic additives and pesticides on nematode multiplication, root-knot index on tomato cv. K-25 inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) under pot conditions^a

Treatments	Final Nematode Population			Repro- duction factor	^b Root- knot index
	Root (Total)	Soil (5Kg)	Total		
Untreated-uninoculated control	--	--	--	--	--
Untreated-inoculated control	25839	32000	57839	11.6	2.85
<i>T. harzianum</i>	10470	16000	26470	5.3	1.25
<i>T. virens</i>	12654	18000	30654	6.1	1.75
<i>P. fluorescens</i>	11526	18000	29526	5.9	1.75
Farm yard manure	24804	30000	55284	11.1	2.60
Neem Seed Powder	9152	12000	21152	4.2	1.00
Carbofuran	4970	8000	12970	2.6	0.75
Topsin-M	20064	26000	49712	9.9	2.50
Bavistin	18780	22000	42658	8.5	2.25
LSD (0.05)	794.55	1096.79	1905.94	0.51	0.13
LSD (0.01)	1082.65	1498.19	2598.75	0.69	0.18

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

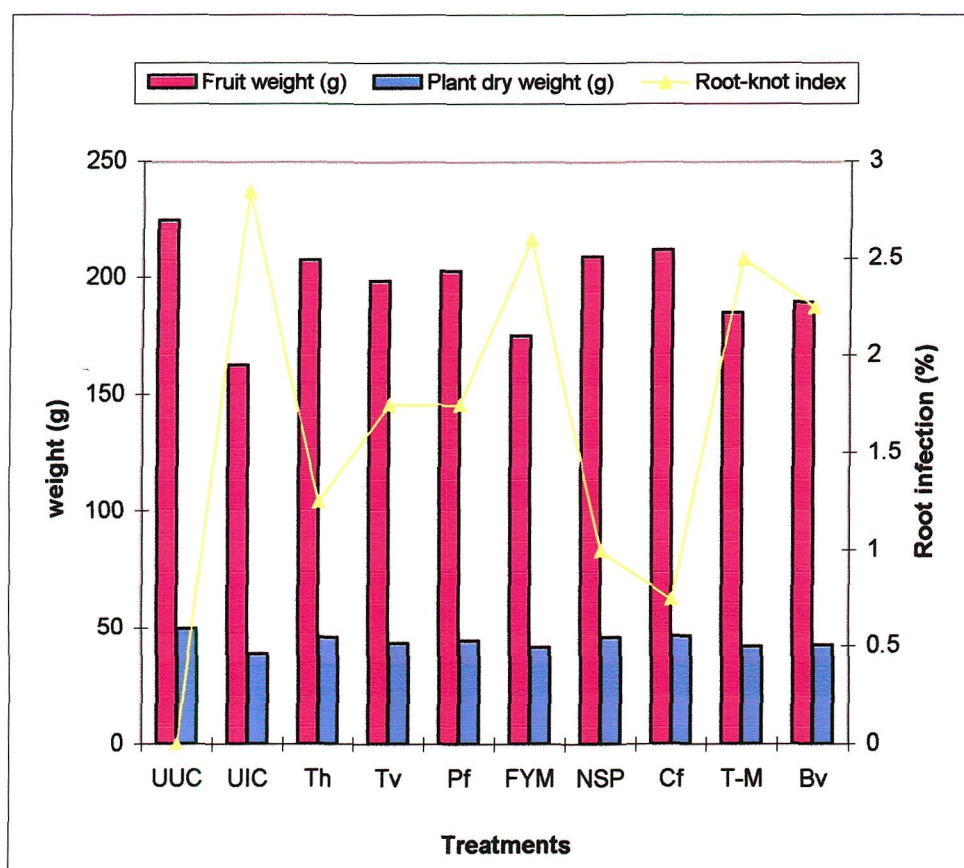


Fig. 8: Comparative efficacy of different additives on root-knot disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *M. incognita* (5000 J₂/5 kg soil) under pot conditions

UUC = Untreated uninoculated control

UIC = Untreated inoculated control

Th = *Trichoderma harzianum*

Tv = *T. virens*

Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure

NSP = Neem seed powder

Cf = Carbofuran

T-M = Topsin-M

Bv = Bavistin

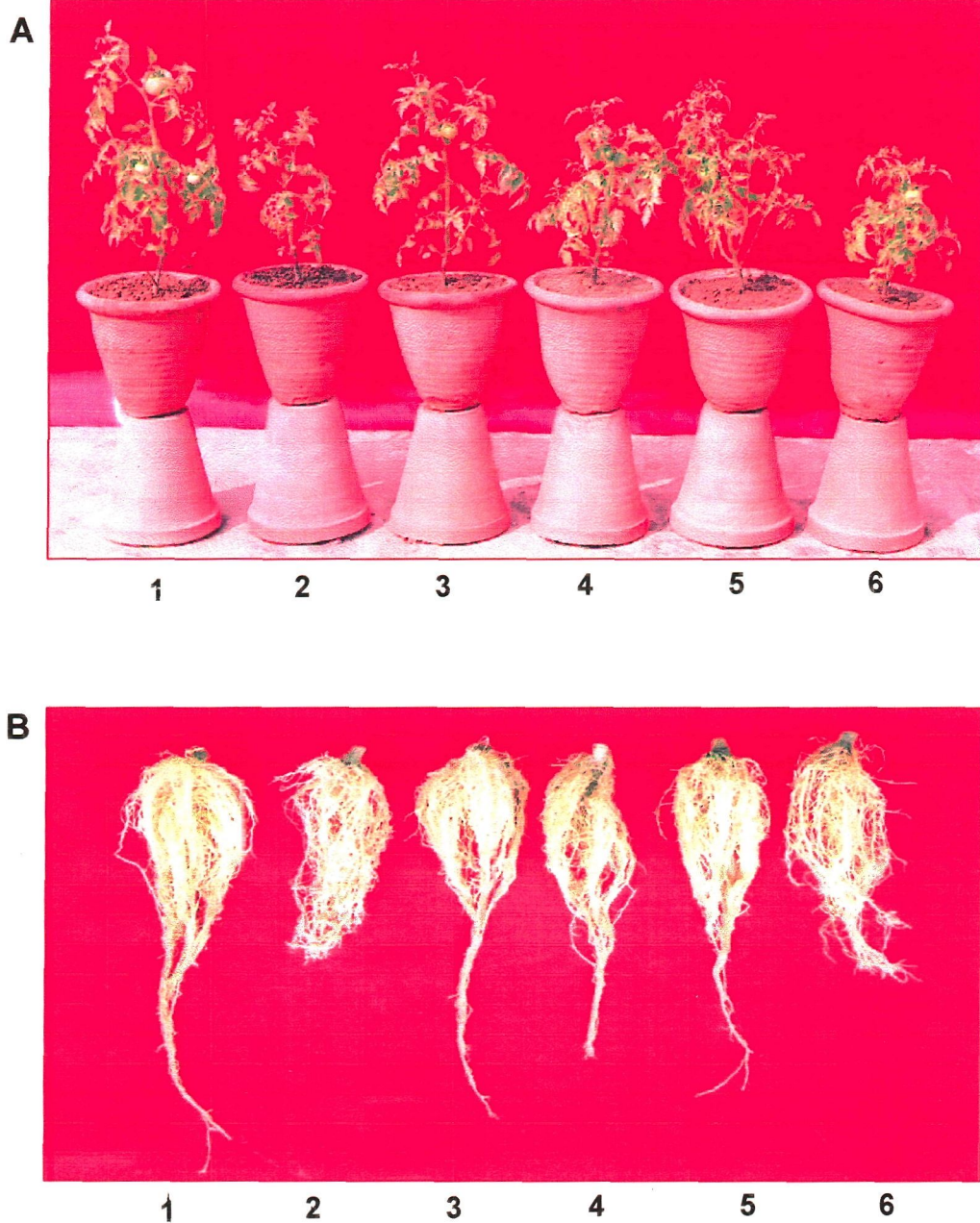


Plate 12 (i): Comparative efficacy of different additives on the aerial growth (A) and root-knot development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil)

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

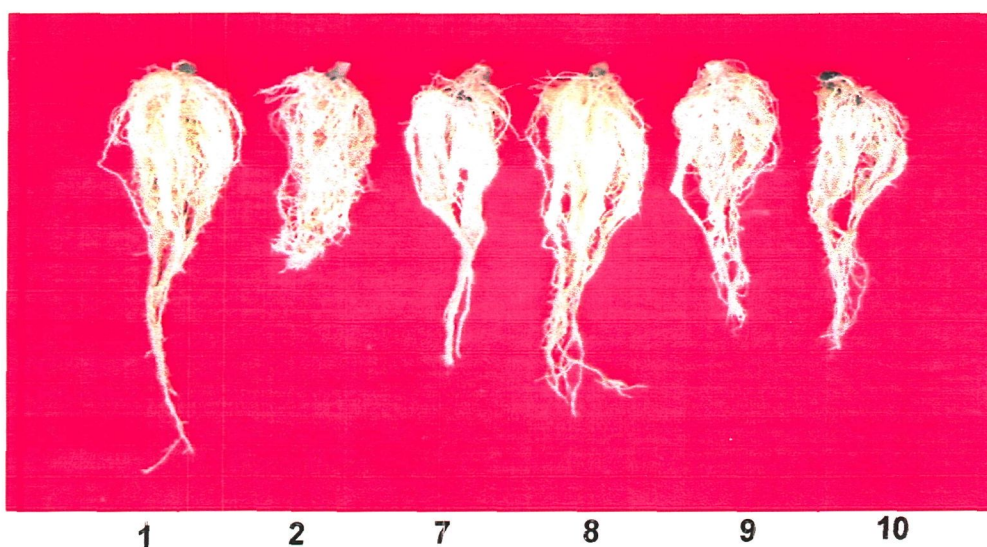
A**B**

Plate 12 (ii): Comparative efficacy of different additives on the aerial growth (A) and root-knot development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil)

1= Uninoculated untreated	8= Carbofuran @ 167 mg/5 kg soil
2= Inoculated untreated	9= Topsin-M @ 12 mg/5 kg soil
7= NSP @ 1250 mg/5 kg soil	10= Bavistin @ 10 mg/5 kg soil

Table 18: Comparative efficacy of biocontrol agents, organic additives and pesticides on disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *Fusarium oxysporum* (7.5×10^6 cfu/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Percent root infection
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total	
Untreated-uninoculated Control	10.4	223.0	45.8	27.9	73.7	175.5	37.5	213.0	38.0	11.5	49.5	-
Untreated inoculated control	8.8 (15.4) ^b	181.0 (18.8)	38.0 (16.6)	24.3 (12.9)	62.3 (15.5)	145.7 (17.0)	32.3 (13.9)	178.0 (16.4)	31.1 (18.2)	9.7 (15.7)	40.8 (17.8)	20.0
<i>T. harzianum</i>	10.2 (1.9)	213.5 (4.3)	44.8 (2.2)	27.4 (1.8)	72.2 (2.0)	170.3 (3.0)	36.7 (2.1)	207.0 (2.8)	36.6 (3.7)	11.1 (3.5)	47.7 (3.6)	3.3
<i>T. virens</i>	9.8 (5.8)	205.5 (7.8)	43.3 (5.5)	26.6 (4.7)	69.9 (5.2)	163.7 (6.7)	35.4 (5.6)	199.1 (6.5)	35.0 (7.9)	10.7 (7.0)	45.7 (7.7)	7.5
<i>P. fluorescens</i>	10.0 (3.8)	210.5 (5.6)	44.2 (3.5)	27.0 (3.2)	71.2 (3.4)	167.3 (4.7)	36.1 (3.7)	203.4 (4.5)	35.9 (5.5)	10.9 (5.2)	46.8 (5.5)	5.5
Farm yard manure	9.2 (11.5)	190.5 (14.7)	39.9 (12.2)	25.2 (9.7)	65.1 (11.7)	153.0 (12.8)	33.7 (10.1)	186.7 (12.3)	32.7 (13.9)	10.1 (12.2)	42.8 (13.5)	17.5
Neem Seed Powder	9.6 (7.7)	200.0 (10.3)	42.0 (8.3)	26.2 (6.1)	68.2 (7.5)	163.3 (7.0)	35.3 (5.9)	198.6 (6.8)	34.9 (8.2)	10.6 (7.8)	45.5 (8.1)	10.0
Carbofuran	9.4 (9.6)	195.3 (12.4)	40.7 (11.1)	25.4 (9.0)	66.1 (10.3)	155.5 (11.4)	33.8 (9.9)	189.3 (11.1)	33.2 (12.6)	10.2 (11.3)	43.4 (12.3)	15.0
Topsin-M	9.8 (5.8)	207.5 (6.9)	43.7 (4.6)	26.9 (3.6)	70.6 (4.2)	164.1 (6.5)	35.5 (5.3)	199.6 (6.3)	35.1 (7.6)	10.7 (7.0)	45.8 (7.5)	6.7
Bavistin	10.0 (3.8)	211.5 (5.2)	44.3 (3.3)	27.3 (2.2)	71.6 (2.8)	169.5 (3.4)	36.5 (2.7)	206.0 (3.3)	36.3 (4.5)	11.0 (4.3)	47.3 (4.4)	5.0
LSD (0.05)	0.37	8.97	1.91	1.16	2.98	7.64	1.46	9.18	1.59	0.38	2.03	0.58
LSD (0.01)	0.50	12.11	2.60	1.58	4.98	10.31	1.97	12.39	2.16	0.51	2.74	0.78

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

^cPercent root infection in root by *F. oxysporum*

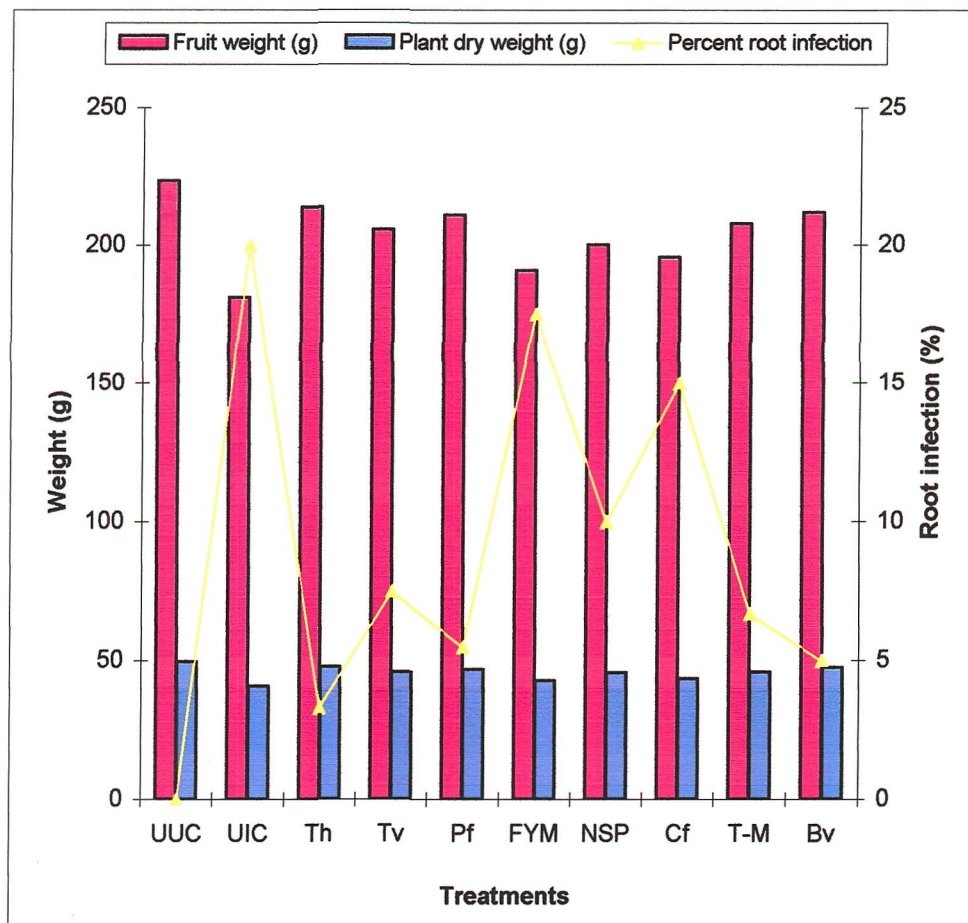


Fig. 9: Comparative efficacy of different additives on disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
UIC = Untreated inoculated control
Th = *Trichoderma harzianum*
Tv = *T. virens*
Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
NSP = Neem seed powder
Cf = Carbofuran
T-M = Topsin-M
Bv = Bavistin

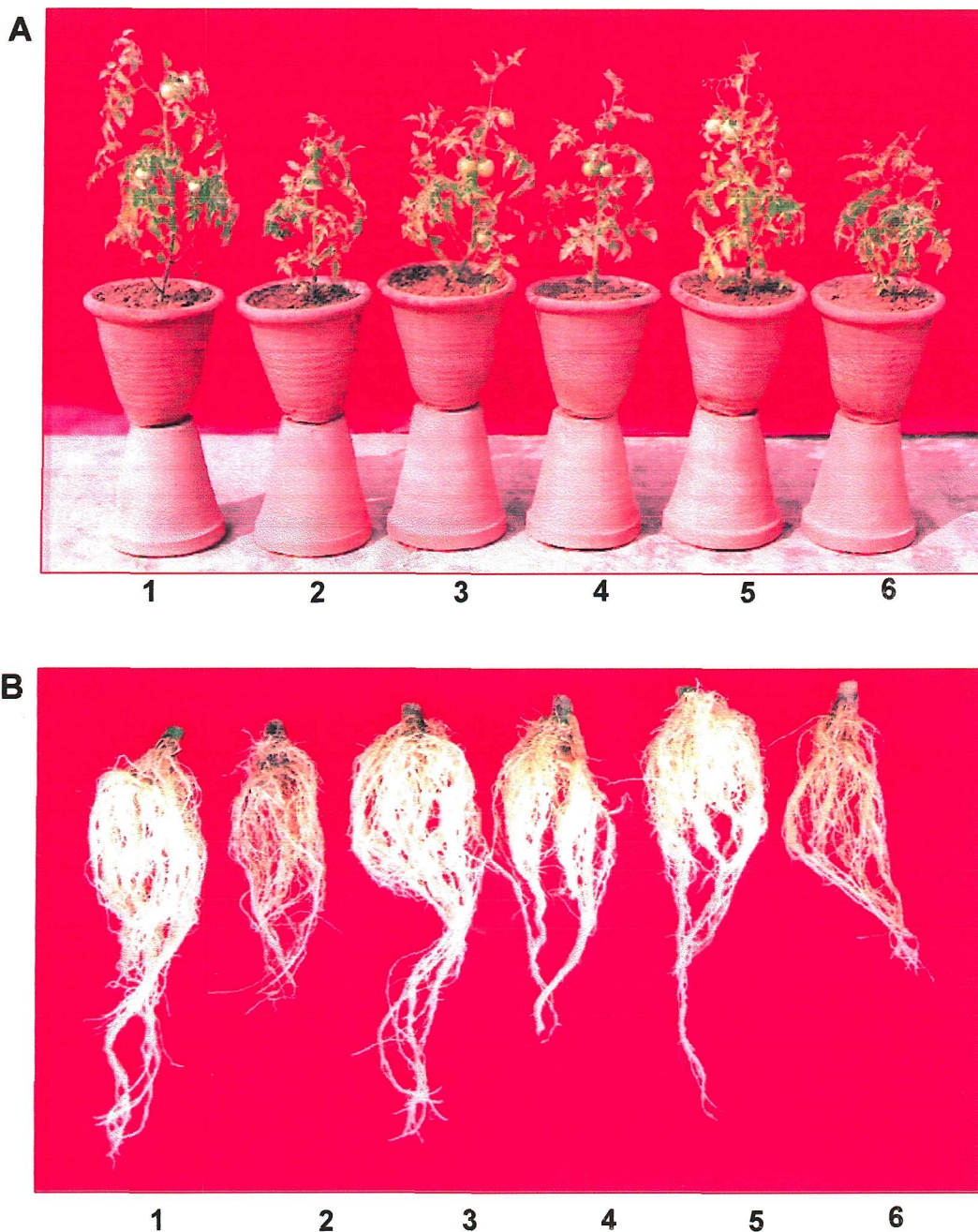


Plate 13 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil)

1= Uninoculated untreated

2= Inoculated untreated

3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil

5= *P. fluorescens* @ 250 mg/5 kg soil

6= FYM @ 7500 mg/5 kg soil

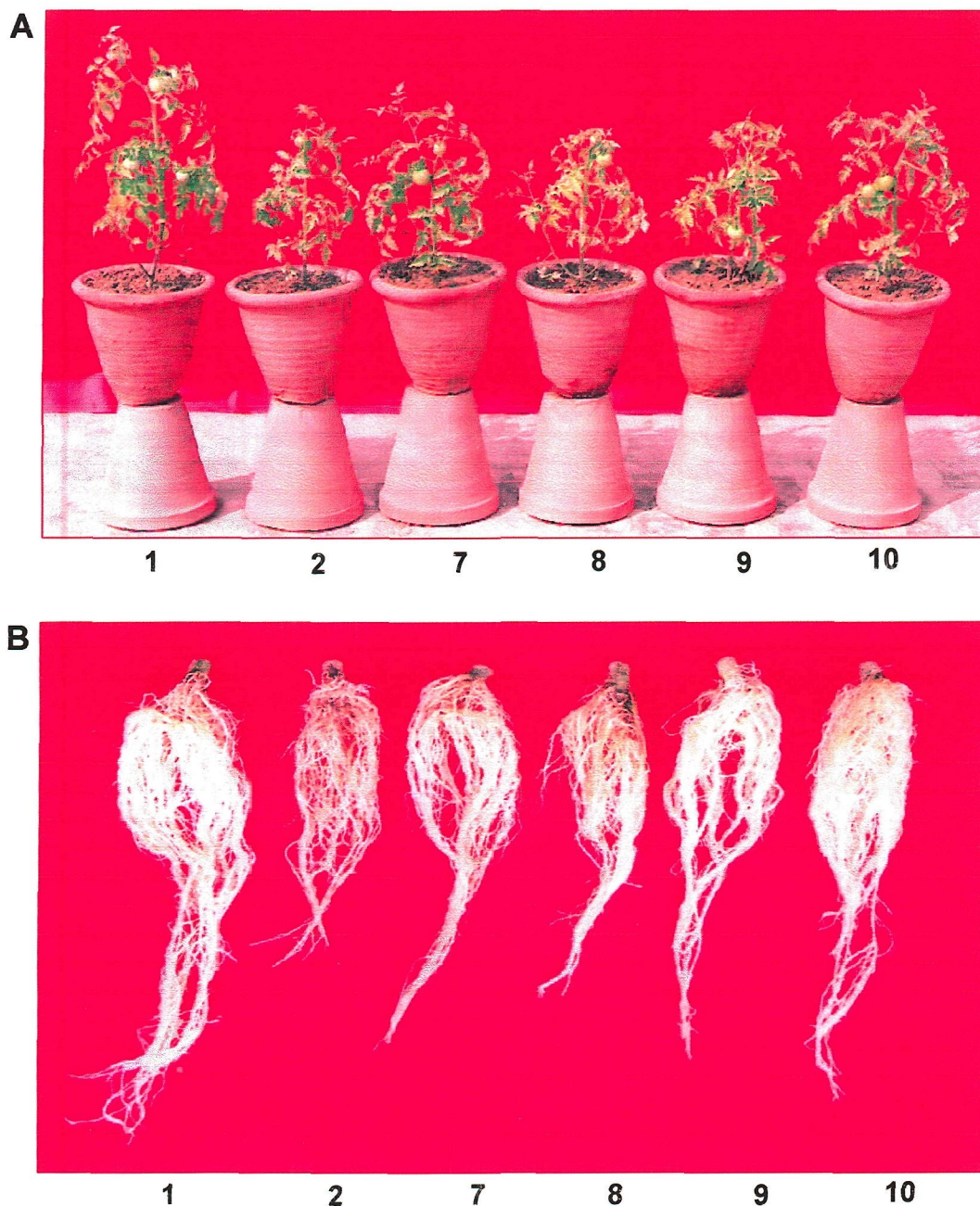


Plate 13 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil)

- | | |
|----------------------------|----------------------------------|
| 1= Uninoculated untreated | 8= Carbofuran @ 167 mg/5 kg soil |
| 2= Inoculated untreated | 9= Topsin-M @ 12 mg/5 kg soil |
| 7= NSP @ 1250 mg/5 kg soil | 10= Bavistin @ 10 mg/5 kg soil |

The maximum reduction in nematode multiplication ($R_f = 2.6$) and root-knot development (0.75) was found in carbofuran treated plants, whereas the highest R_f (11.6) and RKI (2.85) was observed in untreated inoculated plants (Table 17; Fig. 8).

5.2 *F. oxysporum* alone

Data (Table 18) regarding the comparative efficacy of different additives against *F. oxysporum* alone indicated that all the treatments significantly ($P \leq 0.05$) increased the plant growth and fruit yield of tomato cv. K-25 as compared to untreated inoculated plants [Fig. 9; Plate 13 (i), (ii)].

Analyses of data indicated that differences in plant dry weight and fruit yield of tomato plants inoculated with *F. oxysporum* and treated with various additives were mostly significant ($P \leq 0.05$). However, the highest improvement in plant dry weight (47.7 g) and fruit yield (213.5 g) was found in plants treated with *T. harzianum* followed by bavistin, *P. fluorescens*, topsin-M, *T. virens*, neem seed powder, carbofuran, and farmyard manure, respectively.

The extent of root infection by the *F. oxysporum* was decreased by the application of various treatments as compared to untreated inoculated plants. However, the highest reduction in root infection by the fungus (3.3%) was found in plants treated with *T. harzianum* followed by bavistin, *P. fluorescens*, topsin-M, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively (Table 18; Fig. 9).

5.3 *R. solani* alone

Data (Table 19) regarding the comparative efficacy of different additives against *R. solani* indicated that all the treatments significantly ($P \leq 0.05$) increased the plant growth and fruit yield of tomato cv. K-25 as compared to untreated inoculated plants [Fig. 10; Plate 14 (i), (ii)]. Effect of various treatments on plant dry weight and fruit yield was mostly significant ($P \leq 0.05$). However, the highest improvement in plant dry weight (46.2 g) and fruit yield (213.0 g) was found in plants treated with *T. harzianum* followed by topsin-M, *P. fluorescens*, bavistin, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively.

The extent of root infection by the *R. solani* was decreased by the application of various treatments as compared to untreated inoculated plants. The highest reduction in

Table 19: Comparative efficacy of biocontrol agents, organic additives and pesticides on disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)		%Percent root infection		
			Shoot height	Root length	Shoot	Root	Shoot	Root		Total	
Untreated-uninoculated Control	10.6	225.0	46.3	27.6	176.3	37.7	214.0	37.5	10.9	48.4	-
Untreated inoculated control	8.4 (20.8) ^b	173.0 (23.1)	37.0 (20.1)	23.2 (15.9)	140.0 (20.6)	31.1 (17.5)	171.1 (20.0)	29.1 (22.4)	8.9 (18.3)	38.0 (21.5)	25.0
<i>T. harzianum</i>	10.2 (3.8)	213.0 (5.3)	44.8 (3.2)	27.0 (2.2)	169.7 (3.7)	37.3 (1.1)	207.1 (3.2)	35.5 (5.3)	10.7 (1.8)	46.2 (4.5)	5.0
<i>T. vires</i>	9.8 (7.5)	205.5 (8.7)	42.6 (7.8)	26.2 (5.1)	163.9 (7.0)	35.9 (4.8)	199.8 (6.6)	34.2 (8.8)	10.3 (5.5)	44.5 (8.1)	8.5
<i>P. fluorescens</i>	10.0 (5.7)	210.0 (6.7)	43.5 (6.0)	26.4 (4.3)	167.3 (5.1)	36.6 (2.9)	203.9 (4.7)	34.9 (6.9)	10.5 (3.7)	45.4 (6.2)	7.5
Farm yard manure	8.8 (17.0)	183.5 (18.4)	38.8 (16.2)	24.0 (13.0)	149.1 (15.4)	32.8 (13.0)	181.9 (15.0)	31.0 (17.3)	9.4 (13.8)	40.4 (16.5)	21.0
Neem Seed Powder	9.8 (7.5)	203.5 (9.6)	41.6 (10.2)	25.5 (7.6)	161.7 (8.3)	35.7 (5.3)	197.4 (7.8)	33.8 (9.9)	10.2 (6.4)	44.0 (9.1)	11.0
Carbofuran	9.0 (15.1)	185.5 (17.5)	39.0 (15.8)	24.2 (12.3)	152.0 (13.8)	33.5 (11.1)	185.5 (13.3)	31.7 (15.5)	9.6 (11.9)	41.3 (14.7)	20.0
Topsin-M	10.0 (5.7)	211.0 (6.2)	43.7 (5.6)	26.3 (4.7)	168.7 (4.3)	36.9 (2.1)	205.6 (3.9)	35.2 (6.1)	10.6 (2.8)	45.8 (5.4)	6.5
Bavistin	9.8 (7.5)	203.0 (9.8)	42.9 (7.3)	26.0 (5.8)	164.3 (6.8)	36.2 (4.0)	200.5 (6.3)	34.3 (8.5)	10.4 (4.6)	44.7 (7.6)	7.5
LSD (0.05)	0.38	8.93	1.83	1.23	7.83	1.53	9.21	1.69	0.52	2.10	1.00
LSD (0.01)	0.52	12.06	2.49	1.66	10.57	2.07	12.43	2.28	0.70	2.84	1.35

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

^cPercent root infection in root by *R. solani*

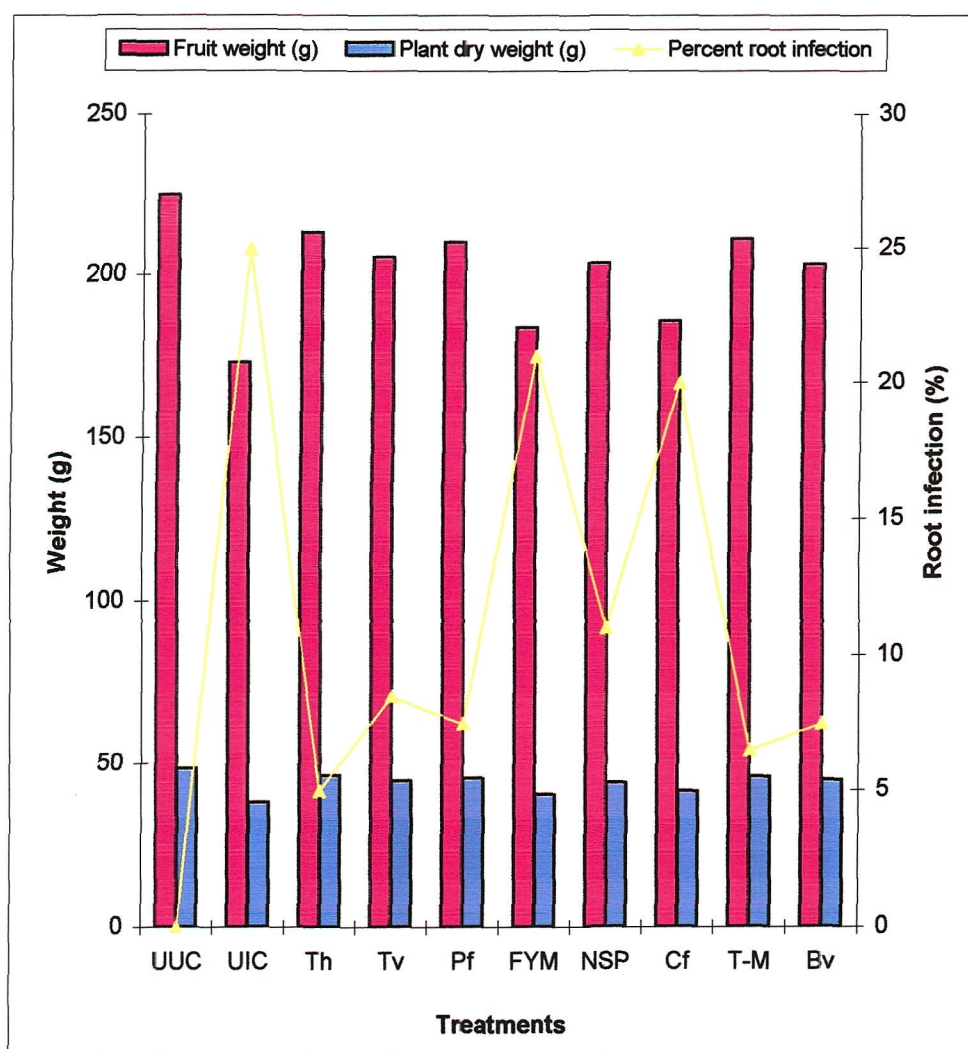


Fig. 10: Comparative efficacy of different additives on disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

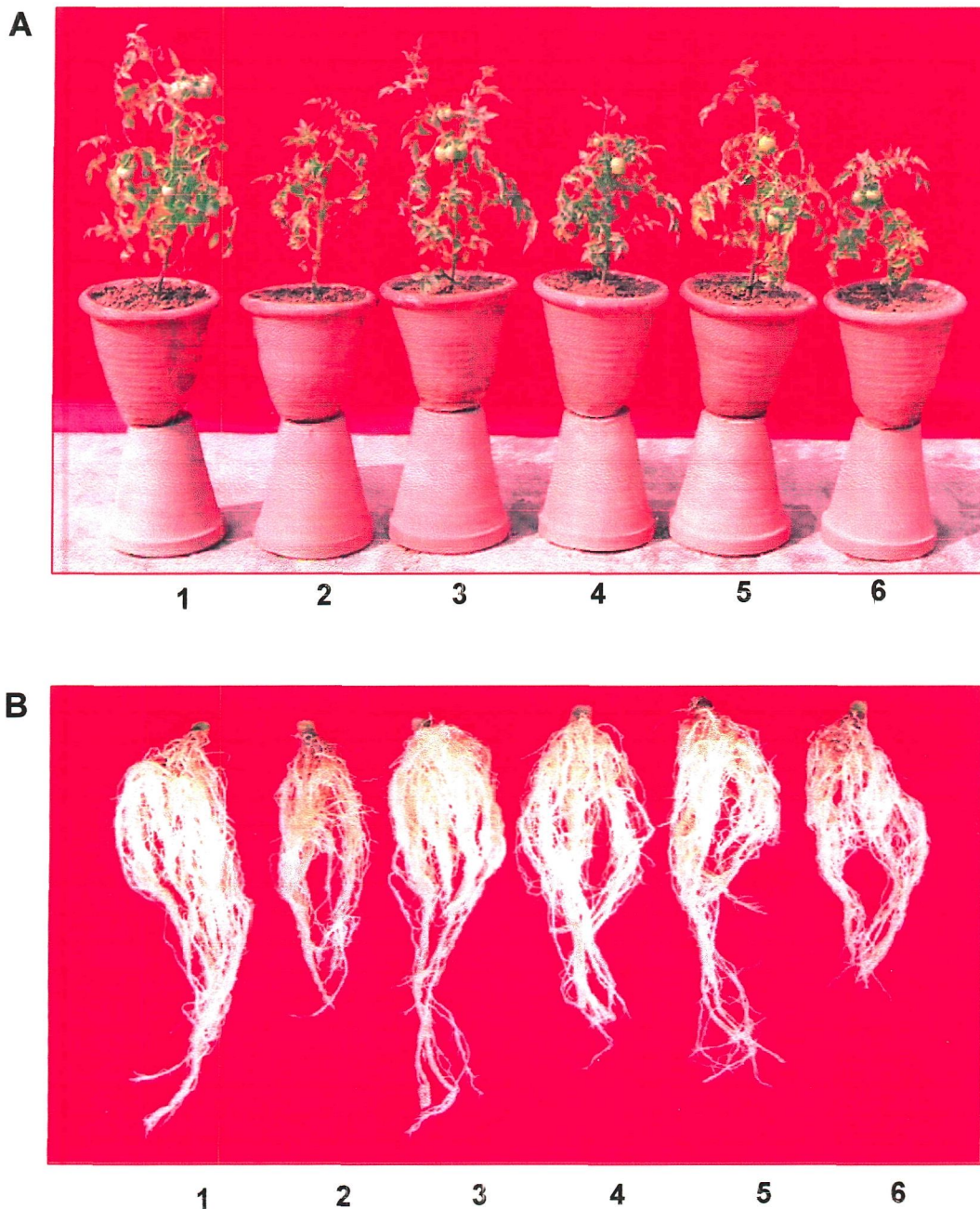


Plate 14 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *R. solani* (7.5 g mycelium/5 kg soil)

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

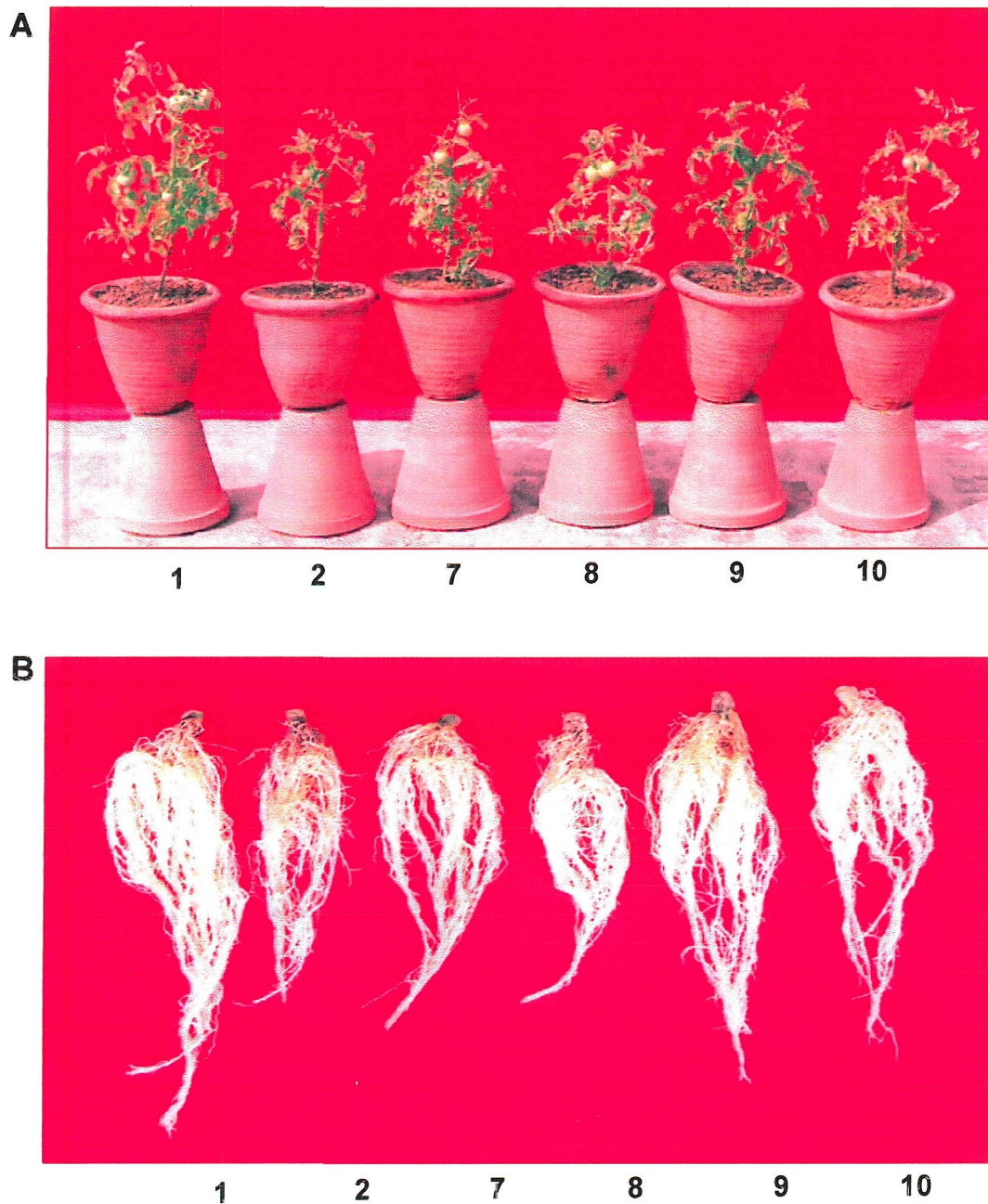


Plate 14 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *R. solani* (7.5 g mycelium/5 kg soil)

1= Uninoculated untreated	8= Carbofuran @ 167 mg/5 kg soil
2= Inoculated untreated	9= Topsin-M @ 12 mg/5 kg soil
7= NSP @ 1250 mg/5 kg soil	10= Bavistin @ 10 mg/5 kg soil

Table 20: Comparative efficacy of biocontrol agents, organic additives and pesticides on disease development, plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *Fusarium oxysporum* (7.5×10^6 cfu/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)		Plant fresh weight (g)		Plant dry weight (g)		%Percent root infection
			Shoot height	Root length	Total	Shoot	Root	Total	
Untreated-uninoculated Control	10.8	224.0	45.6	27.9	73.5	174.5	37.0	211.5	48.4
Untreated inoculated control	6.0 (44.4) ^b	120.0 (46.4)	29.5 (35.3)	17.3 (38.0)	46.8 (36.3)	103.3 (40.8)	20.8 (43.8)	124.1 (41.3)	27.4 (43.4)
<i>T. harzianum</i>	10.0 (7.4)	205.0 (8.5)	43.5 (4.6)	26.0 (6.8)	69.5 (5.4)	165.7 (5.0)	34.2 (7.6)	199.9 (5.5)	9.7 (9.3)
<i>T. virens</i>	9.6 (11.1)	195.0 (12.9)	42.8 (6.1)	25.5 (8.6)	68.3 (7.1)	160.7 (7.9)	33.1 (10.5)	193.8 (8.4)	9.4 (12.1)
<i>P. fluorescens</i>	9.8 (9.3)	197.5 (11.8)	43.0 (5.7)	25.7 (7.9)	68.7 (6.5)	164.3 (5.8)	33.9 (8.4)	198.2 (6.3)	9.6 (10.3)
Farm yard manure	7.4 (31.5)	150.0 (33.0)	34.2 (25.0)	20.2 (27.6)	54.4 (26.0)	124.5 (28.7)	25.3 (31.6)	149.8 (29.2)	26.2 (30.5)
Neem Seed Powder	9.0 (16.7)	183.0 (18.3)	38.8 (14.9)	23.1 (17.2)	61.9 (15.8)	138.7 (20.5)	28.4 (23.2)	167.1 (21.0)	29.3 (22.3)
Carbofuran	8.4 (22.2)	172.0 (23.2)	35.7 (21.7)	21.1 (24.4)	56.8 (22.7)	131.7 (24.5)	26.9 (27.3)	158.6 (25.0)	27.8 (26.3)
Topsin-M	9.4 (13.0)	190.0 (15.2)	42.6 (6.6)	25.4 (9.0)	68.0 (7.5)	160.0 (8.3)	32.9 (11.1)	192.9 (8.9)	33.9 (10.1)
Bavistin	9.8 (9.3)	200.0 (10.7)	43.1 (5.5)	25.7 (7.9)	68.8 (6.4)	164.7 (5.6)	33.9 (8.4)	198.7 (6.1)	34.9 (7.4)
LSD (0.05)	0.36	9.45	1.97	1.31	3.47	7.49	1.51	9.66	1.49
LSD (0.01)	0.48	12.77	2.66	1.77	4.68	10.11	2.04	13.04	2.01
									0.59
									2.78

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

^cPercent root infection in root by *F. Oxysporum* and *R. solani*

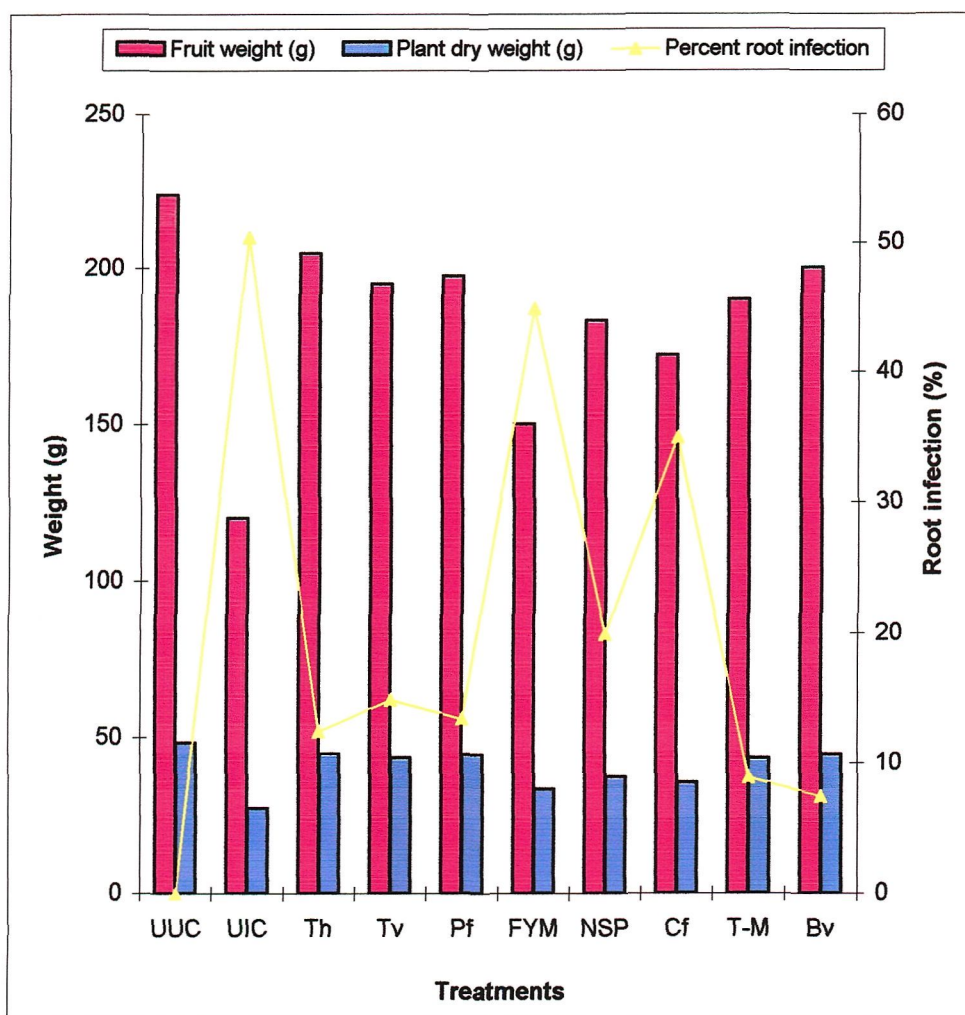


Fig. 11: Comparative efficacy of different additives on disease development, plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

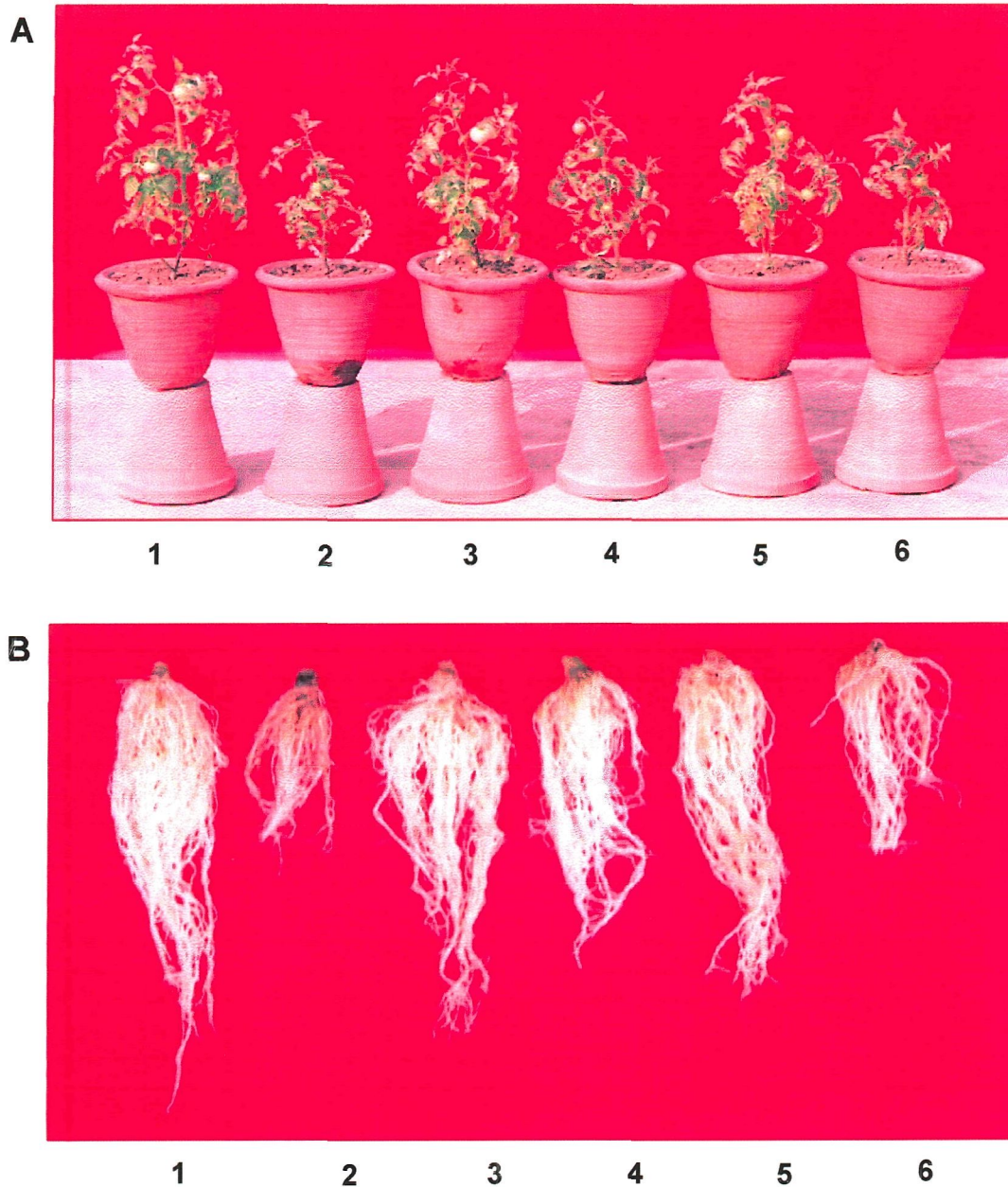


Plate 15 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

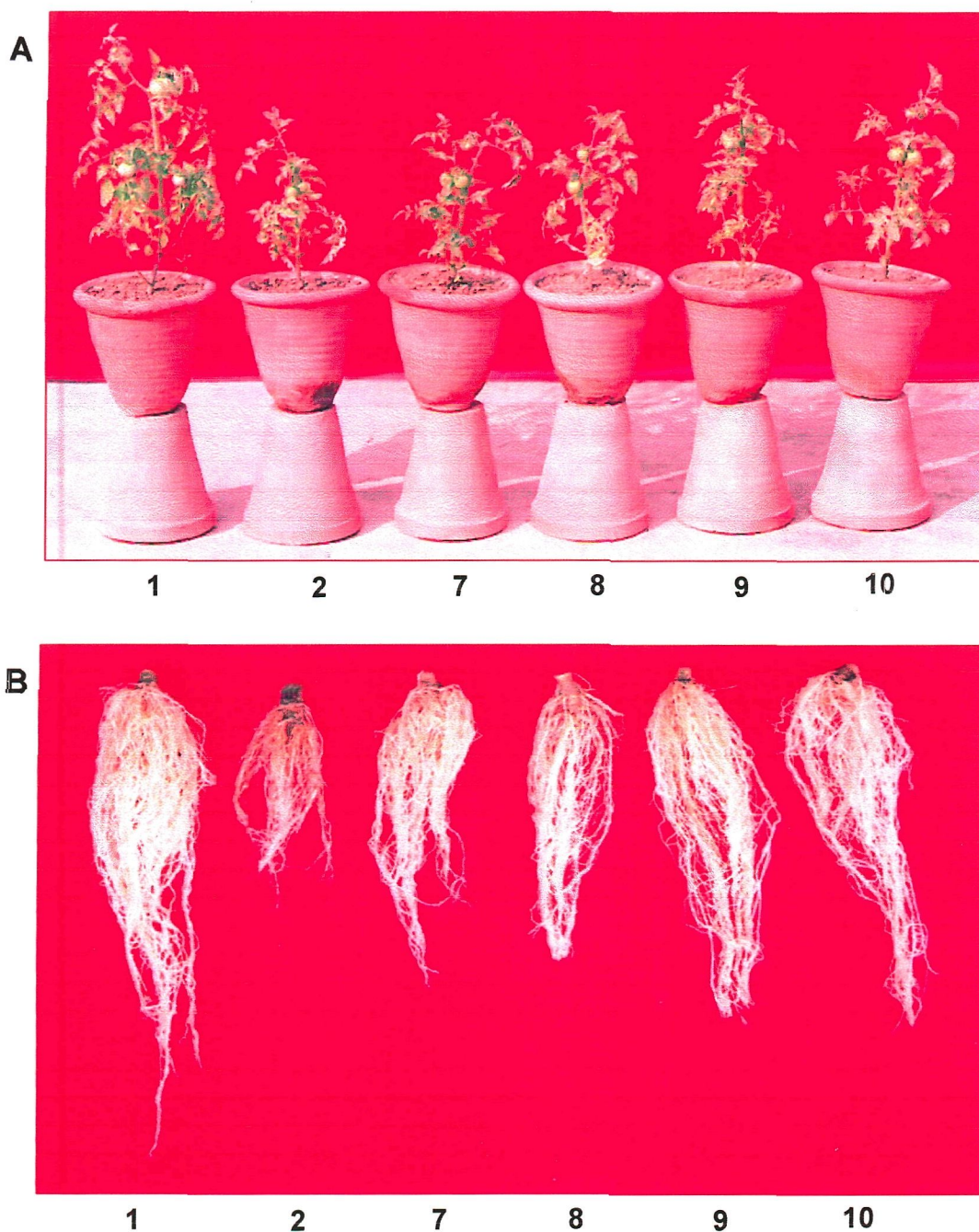


Plate 15 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *F. oxysporum* (7.5×10^6 cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 7= NSP @ 1250 mg/5 kg soil

8= Carbofuran @ 167 mg/5 kg soil
 9= Topsin-M @ 12 mg/5 kg soil
 10= Bavistin @ 10 mg/5 kg soil

root infection by the fungus (5.0%) was noted in plants treated with *T. harzianum* followed by topsin-M, bavistin, *P. fluorescens*, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively (Table 19; Fig. 10).

5.4 *F. oxysporum* and *R. solani* simultaneously

Studies regarding the comparative efficacy of different additives against *F. oxysporum* and *R. solani* simultaneously inoculated plants of tomato cv. K-25 indicated that in general, application of all the treatments were able to increase the plant growth and fruit yield as compared to untreated inoculated plants [Table 20; Fig. 11; Plate 15 (i), (ii)]. All the treatments were also able to reduce the root infection by *F. oxysporum* and *R. solani*.

Statistical analyses of data indicated that the differences in plant dry weight and fruit yield of tomato plants inoculated with *F. oxysporum* and *R. solani* and treated with various additives were mostly significant ($P \leq 0.05$). However, the highest improvement in plant dry weight (44.9 g) and fruit yield (205.0 g) was found in plants treated with *T. harzianum* followed by bavistin, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, carbofuran and farmyard manure, respectively as compared to untreated inoculated plants. The highest reduction in root infection due to *F. oxysporum* and *R. solani* (7.5%) was found in bavistin treated plants followed by topsin-M, *T. harzianum*, *P. fluorescens*, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively (Table 20; Fig. 11).

5.5 *M. incognita* and *F. oxysporum* simultaneously

Data (Table 21) regarding the comparative efficacy of different additives against *M. incognita* and *F. oxysporum* simultaneously inoculated plants of tomato cv. K-25 indicated that in general, application of all the treatments were able to increase the plant growth and fruit yield as compared to untreated inoculated plants [Fig. 12; Plate 16 (i), (ii)].

Analyses of data indicated that differences on the effect of various treatments on dry weight and fruit yield of tomato plants were mostly significant ($P \leq 0.05$). However, highest improvement in plant dry weight (42.7 g) and fruit yield (202.5 g) was found in plants treated with *T. harzianum* followed by carbofuran, *P. fluorescens*, bavistin, *T.*

Table 21: Comparative efficacy of biocontrol agents, organic additives and pesticides on plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) and *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total
Untreated-uninoculated Control	10.6	223.5	45.2	27.8	73.0	173.7	36.5	210.2	37.3	10.5	47.8
Untreated inoculated control	5.4 (49.1) ^b	110.0 (50.8)	27.0 (40.3)	15.7 (43.5)	42.7 (41.5)	93.5 (46.2)	18.1 (50.4)	111.6 (46.9)	19.3 (48.4)	4.9 (53.3)	24.1 (49.6)
<i>T. harzianum</i>	9.8 (7.5)	202.5 (9.4)	41.8 (7.5)	25.1 (9.7)	66.9 (8.4)	158.5 (8.8)	32.3 (11.5)	190.8 (9.2)	33.7 (9.7)	9.0 (14.3)	42.7 (10.7)
<i>T. virens</i>	9.2 (13.2)	192.5 (13.9)	40.0 (11.5)	24.0 (13.7)	64.0 (12.3)	152.9 (12.0)	31.1 (14.8)	184.0 (12.5)	32.1 (13.9)	8.7 (17.1)	40.8 (14.6)
<i>P. fluorescens</i>	9.4 (11.3)	193.5 (13.4)	40.5 (10.4)	24.2 (12.9)	64.7 (11.4)	154.5 (11.1)	31.5 (13.7)	186.0 (11.5)	32.5 (12.9)	8.9 (15.2)	41.4 (13.4)
Farm yard manure	6.0 (43.4)	122.5 (45.2)	30.3 (33.0)	17.7 (36.3)	48.0 (34.2)	108.3 (37.7)	21.7 (40.5)	130.0 (38.2)	22.5 (39.7)	5.9 (43.8)	28.4 (40.6)
Neem Seed Powder	8.8 (17.0)	185.0 (17.2)	39.5 (12.6)	23.6 (15.1)	63.1 (13.6)	147.7 (15.0)	29.9 (18.1)	177.6 (15.5)	30.9 (17.2)	8.3 (20.9)	39.2 (18.0)
Carbofuran	9.6 (9.4)	197.5 (11.6)	41.5 (8.2)	24.7 (11.2)	66.2 (9.3)	155.5 (10.5)	31.7 (13.2)	187.2 (10.9)	32.7 (12.3)	8.9 (15.2)	41.6 (13.0)
Topsin-M	9.0 (15.1)	180.0 (19.5)	39.0 (13.7)	23.3 (16.2)	62.3 (14.7)	144.3 (16.9)	29.3 (19.7)	173.6 (17.4)	30.3 (18.8)	8.1 (22.9)	38.4 (19.7)
Bavistin	9.2 (13.2)	192.0 (14.1)	40.3 (10.8)	24.0 (13.7)	64.3 (11.9)	154.1 (11.3)	31.3 (14.2)	185.4 (11.8)	32.3 (13.4)	8.7 (17.1)	41.0 (14.2)
LSD (0.05)	0.29	9.72	2.03	1.34	3.29	7.78	1.62	9.21	1.64	0.46	2.15
LSD (0.01)	0.39	13.10	2.74	1.81	4.43	10.49	2.18	12.41	2.21	0.62	2.90

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

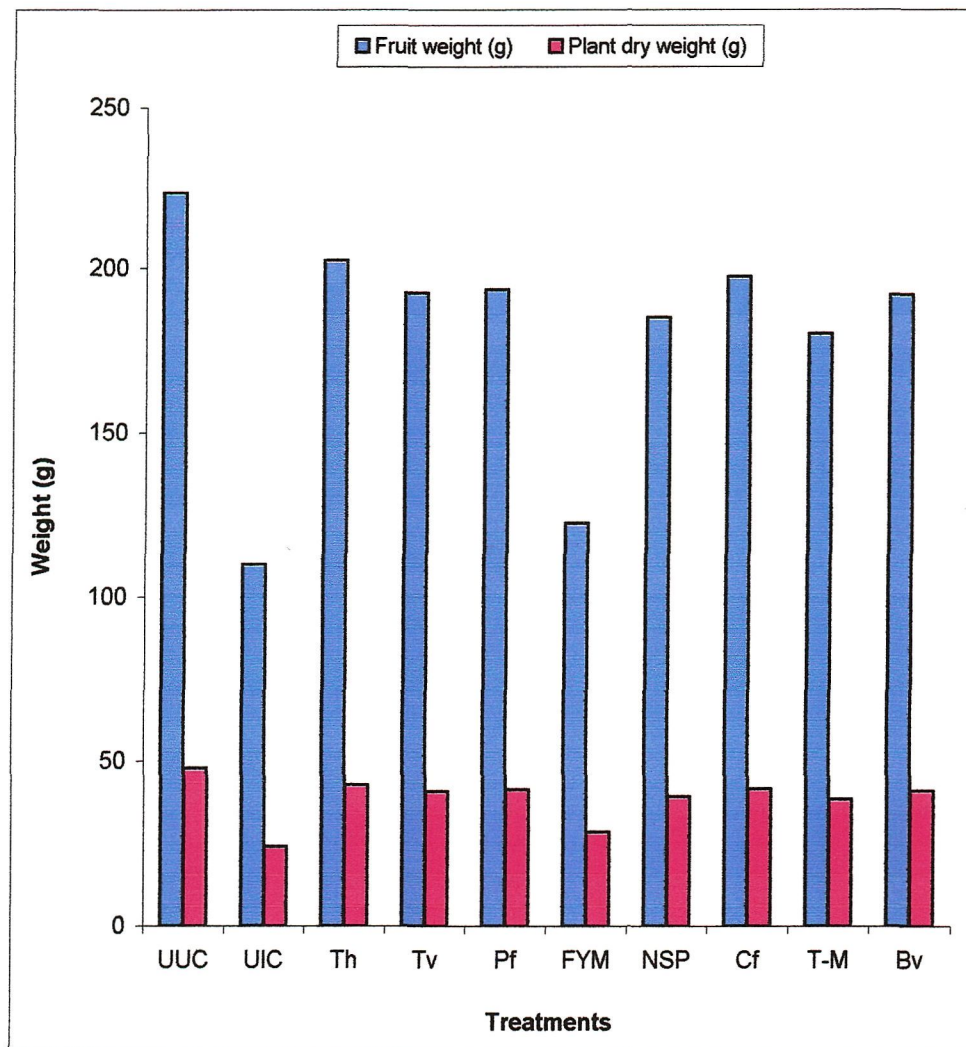


Fig. 12: Comparative efficacy of different additives on plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil) and *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

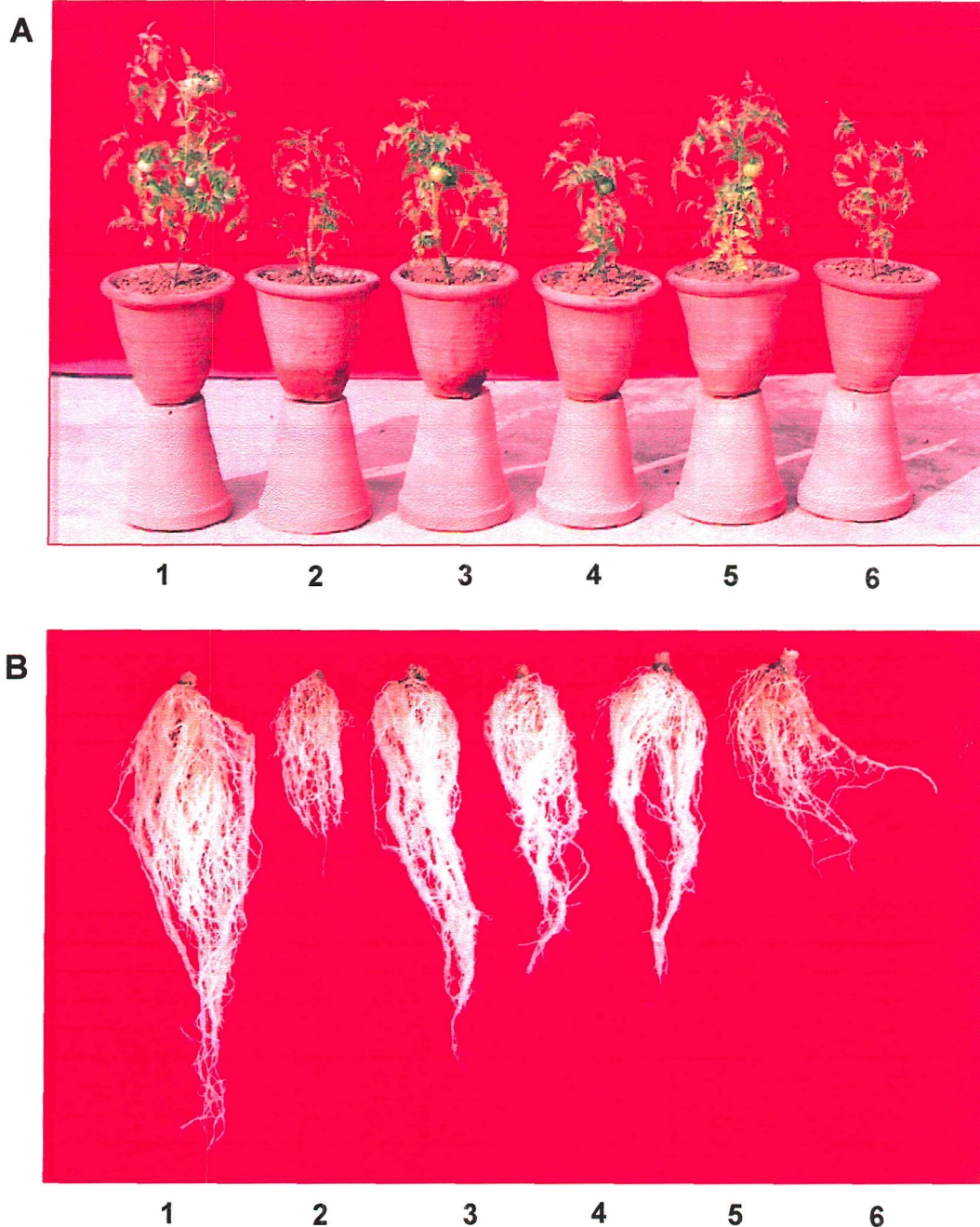


Plate 16 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil) and *F. oxysporum* (7.5×10^6 cfu/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

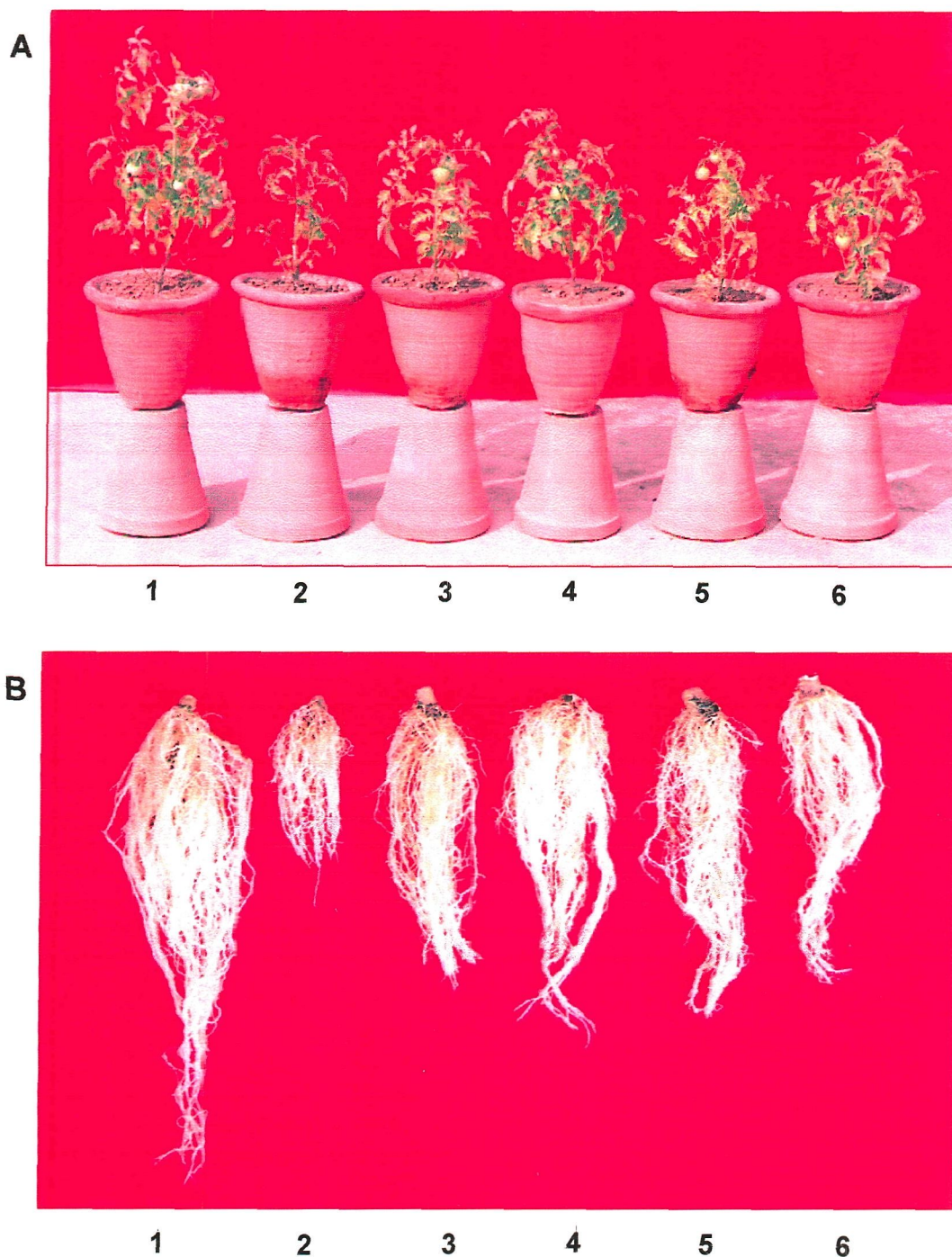


Plate 16 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil) and *F. oxysporum* (7.5×10^6 cfu/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 7= NSP @ 1250 mg/5 kg soil

8= Carbofuran @ 167 mg/5 kg soil
 9= Topsin-M @ 12 mg/5 kg soil
 10= Bavistin @ 10 mg/5 kg soil

Table 22:

Comparative efficacy of different biocontrol agents, organic additives and pesticides on nematode multiplication, root-knot index and percent root infection by fungus in tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) and *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) under pot conditions^a

Treatments	Final nematode population			Reproduction factor	^b Root-knot index	^c Percent root infection
	Root (Total)	Soil (5 Kg)	Total			
Untreated-uninoculated control	--	--	--	--	--	--
Untreated-inoculated control	11222	20000	31222	6.2	2.30	45.0
<i>T. harzianum</i>	7752	8000	15752	3.2	1.25	7.5
<i>T. virens</i>	9952	12000	21952	4.4	1.60	13.0
<i>P. fluorescens</i>	8820	10000	18820	3.8	1.45	13.5
Farm yard manure	11284	18000	29284	5.9	2.25	40.0
Neem Seed Powder	5980	10000	15980	3.2	1.25	20.0
Carbofuran	2536	4000	6536	1.3	0.50	12.5
Topsin-M	11134	16000	27134	5.4	2.00	10.0
Bavistin	10016	14000	24016	4.8	1.75	5.0
LSD (0.05)	428.07	622.05	1085.82	0.21	0.14	1.97
LSD (0.01)	584.70	849.26	1479.41	0.28	0.18	2.66

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by *F. oxysporum*

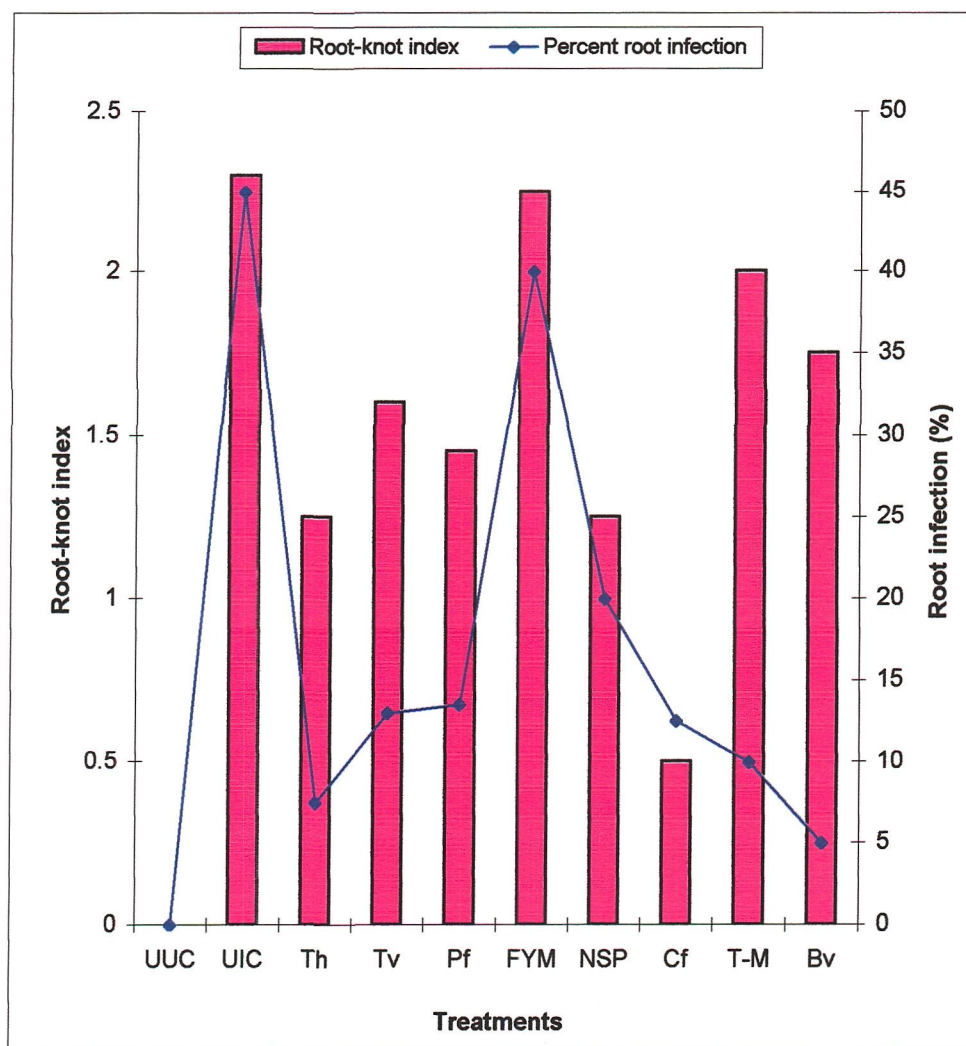


Fig. 13: Comparative efficacy of different additives on disease development in tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil) and *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

virens, neem seed powder, topsin-M and farmyard manure, respectively as compared to untreated inoculated plants.

Data presented in (Table 22) indicated that all the treatments were also able to reduce the root-knot development, *M. incognita* reproduction rate and percent root infection by the *F. oxysporum*. The highest reduction in nematode Rf (1.3) and RKI (0.50) was found in carbofuran treated plants followed by *T. harzianum*, neem seed powder, *P. fluorescens*, *T. virens*, bavistin, topsin-M and farmyard manure, respectively [Fig. 13; Plate 16 (i), (ii)]. The highest reduction in root infection (5.0%) due to *F. oxysporum* was found in bavistin treated plants followed by *T. harzianum*, topsin-M, carbofuran, *T. virens*, *P. fluorescens*, neem seed powder and farmyard manure, respectively. The highest Rf (6.2), RKI (2.30) and PRI (45.0) was found in untreated inoculated plants.

5.6 *M. incognita* and *R. solani* simultaneously

Data presented in (Table 23) regarding the comparative efficacy of different additives against *M. incognita* and *R. solani* simultaneously inoculated plants of tomato cv. K-25 indicated that in general, application of all the treatments were able to increase the plant growth and fruit yield of the plants as compared to untreated inoculated plants [Fig. 14; Plate 17 (i), (ii)].

Analyses of data indicated that the differences on the effect of various treatments on plant dry weight and fruit yield of tomato were mostly significant ($P \leq 0.05$). However, the highest improvement in plant dry weight (42.4 g) and fruit yield (197.5 g) was found in plants treated with *T. harzianum* followed by carbofuran, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, bavistin and farmyard manure, respectively as compared to untreated inoculated plants.

All the treatments were also able to reduce the root-knot development, *M. incognita* reproduction rate and percent root infection by the *R. solani*. The highest reduction in nematode Rf (1.6) and RKI (0.60) was found in carbofuran treated plants followed by *T. harzianum*, neem seed powder, *P. fluorescens*, *T. virens*, bavistin, topsin-M and farmyard manure, respectively. The highest reduction in root infection (7.5%) due to *R. solani* was found in topsin-M treated plants followed by *T. harzianum*, bavistin,

Table 23: Comparative efficacy of biocontrol agents, organic additives and pesticides on plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total
Untreated-uninoculated Control	10.8	225.0	45.6	27.9	73.5	174.0	36.9	210.9	37.5	10.9	48.4
Untreated inoculated control	4.6 (57.4) ^b	92.5 (58.9)	24.8 (45.6)	14.3 (48.7)	39.1 (46.8)	85.3 (51.0)	16.7 (54.7)	102.0 (51.6)	17.5 (53.3)	4.7 (56.90)	22.2 (54.1)
<i>T. harzianum</i>	9.8 (10.2)	197.5 (12.2)	41.5 (9.0)	24.6 (11.8)	66.1 (10.1)	157.5 (9.5)	32.3 (12.5)	189.8 (10.0)	33.1 (11.7)	9.3 (14.7)	42.4 (12.4)
<i>T. virens</i>	9.2 (14.8)	187.5 (16.7)	40.0 (12.3)	23.7 (15.1)	63.7 (13.3)	152.3 (12.5)	31.3 (15.2)	183.6 (12.9)	32.1 (14.4)	9.0 (17.4)	41.1 (15.1)
<i>P. fluorescens</i>	9.4 (13.0)	190.0 (15.6)	40.4 (11.4)	23.9 (14.3)	64.3 (12.5)	154.0 (11.5)	31.5 (14.6)	185.5 (12.0)	32.5 (13.3)	9.1 (16.5)	41.6 (14.0)
Farm yard manure	6.0 (44.4)	120.0 (46.7)	28.7 (37.1)	16.6 (40.5)	45.3 (38.4)	105.3 (39.5)	21.0 (43.1)	126.3 (40.1)	21.9 (41.6)	5.9 (45.9)	27.8 (42.6)
Neem Seed Powder	8.8 (18.5)	177.5 (21.1)	39.6 (13.2)	23.4 (16.1)	63.0 (14.3)	146.3 (15.9)	30.0 (18.2)	176.2 (16.5)	30.7 (18.1)	8.7 (20.2)	39.4 (18.6)
Carbofuran	9.6 (11.1)	195.0 (13.3)	41.3 (9.4)	24.5 (12.2)	65.8 (10.5)	154.9 (11.0)	31.6 (14.4)	186.5 (11.6)	32.5 (13.3)	9.1 (16.5)	41.6 (14.0)
Topsin-M	9.0 (16.7)	182.5 (18.9)	40.1 (12.1)	23.6 (15.4)	63.7 (13.3)	149.5 (14.1)	30.9 (16.3)	180.4 (14.5)	31.3 (16.5)	8.7 (18.3)	40.0 (17.4)
Bavistin	8.6 (20.3)	175.0 (22.2)	39.5 (13.4)	23.3 (16.5)	62.8 (14.6)	143.7 (17.4)	29.3 (20.6)	173.0 (18.0)	30.1 (19.7)	8.5 (22.0)	38.6 (20.2)
LSD (0.05)	0.33	10.13	1.99	1.23	3.30	7.62	1.52	9.10	1.43	0.52	2.23
LSD (0.01)	0.45	13.66	2.67	1.66	4.44	10.28	2.05	12.30	1.93	0.70	3.00

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

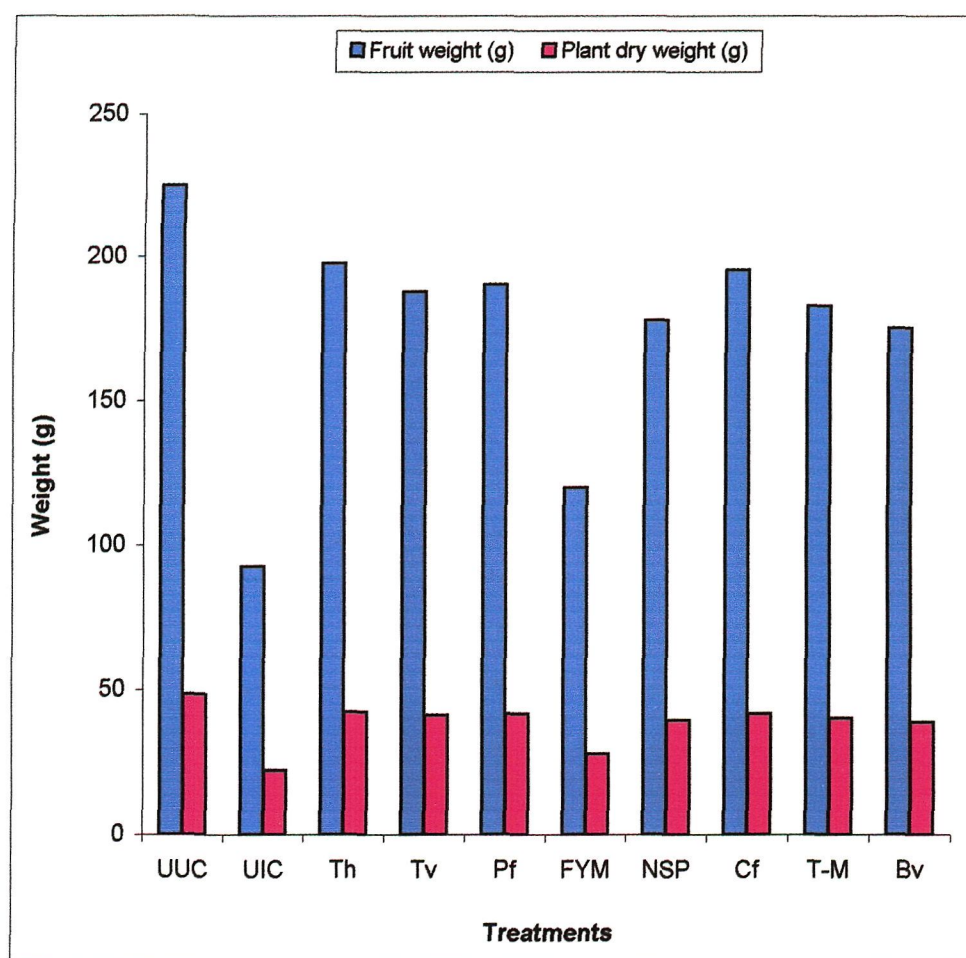


Fig. 14: Comparative efficacy of different additives on plant growth and fruit yield in tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

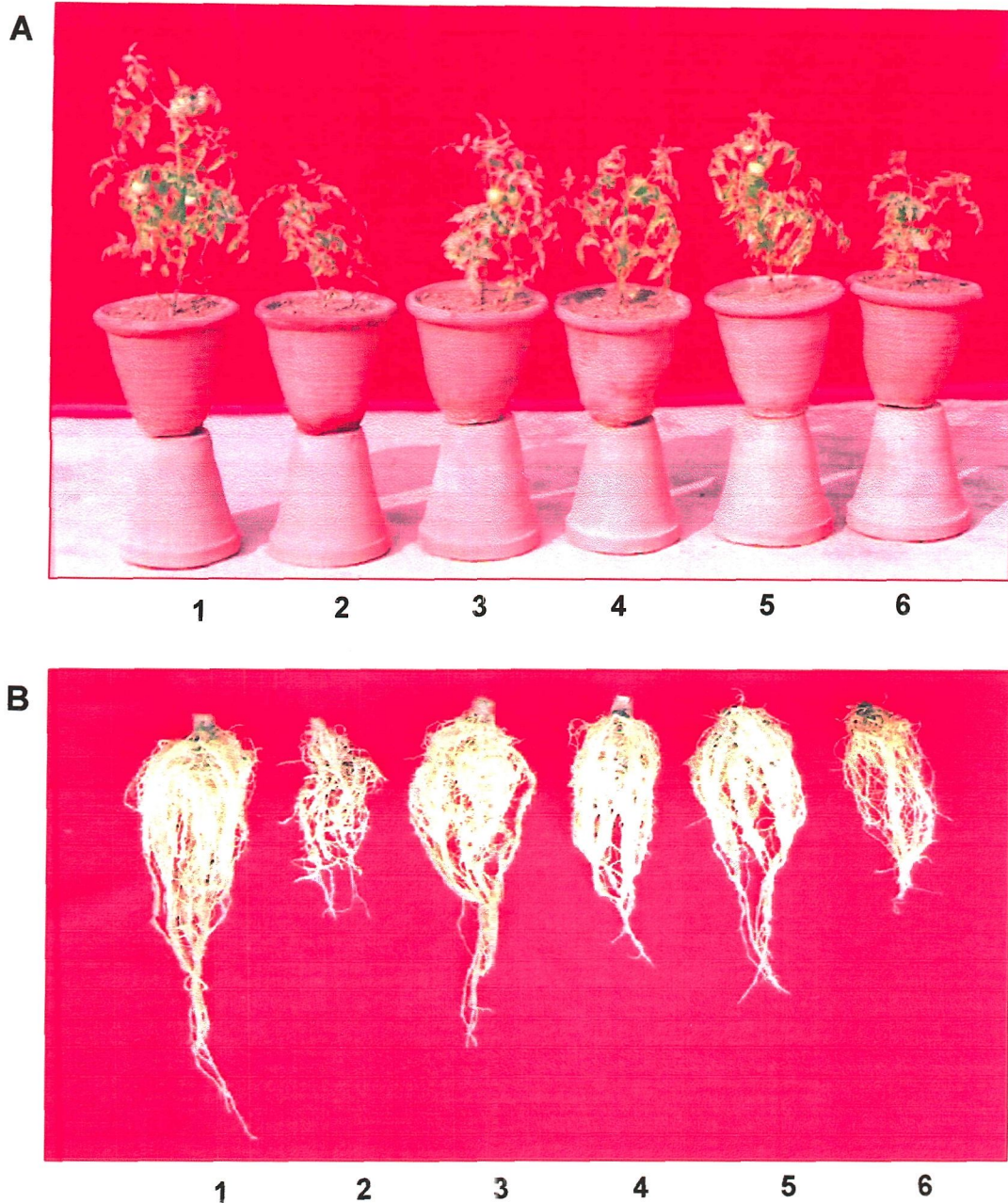
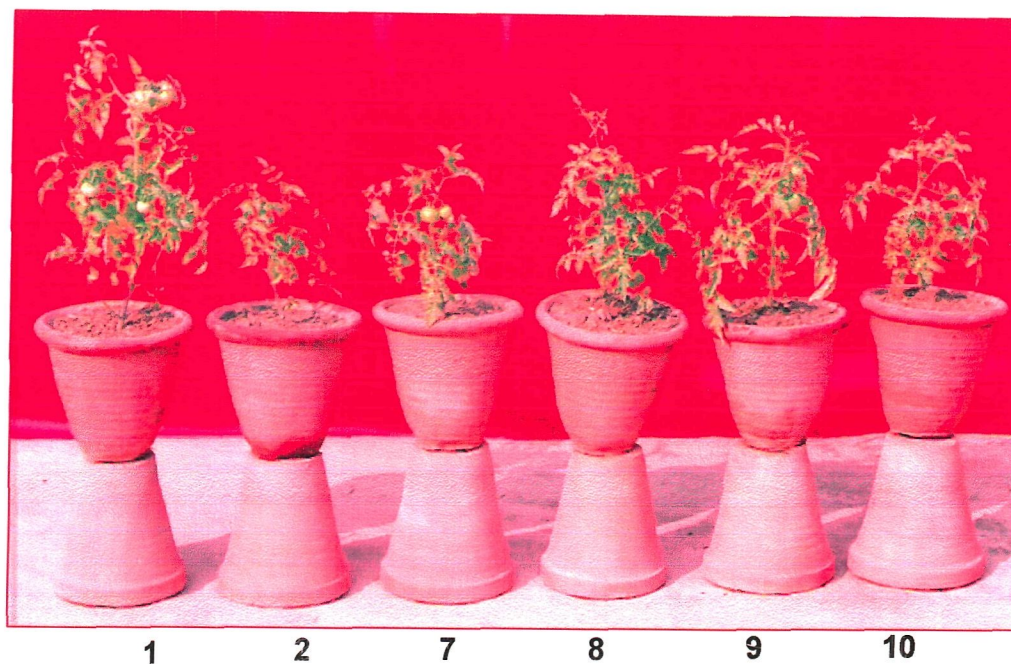


Plate 17 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

A



B

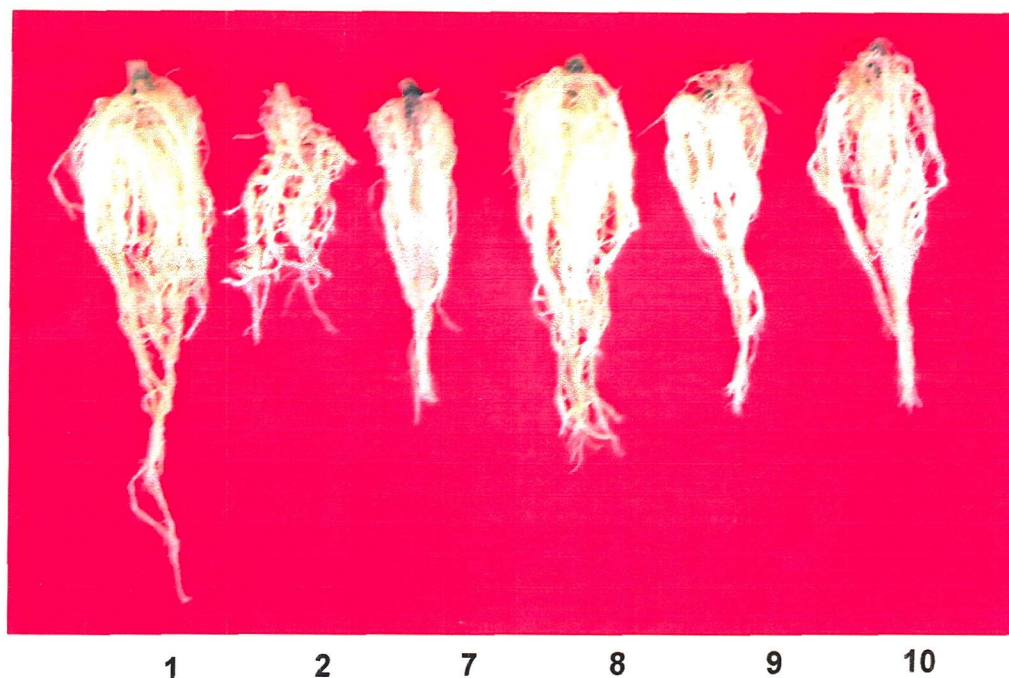


Plate 17 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
2= Inoculated untreated
7= NSP @ 1250 mg/5 kg soil

8= Carbofuran @ 167 mg/5 kg soil
9= Topsin-M @ 12 mg/5 kg soil
10= Bavistin @ 10 mg/5 kg soil

Table 24:

Comparative efficacy of different biocontrol agents, organic additives and pesticides on nematode multiplication, root-knot index and percent root infection by fungus in tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Final nematode population			Reproduction factor	^b Root-knot index	^c Percent root infection
	Root (Total)	Soil (5 Kg)	Total			
Untreated-uninoculated control	--	--	--	--	--	--
Untreated-inoculated control	12358	22000	34358	6.9	2.50	57.5
<i>T. harzianum</i>	8398	10000	18398	3.7	1.50	9.0
<i>T. virens</i>	10016	14000	24016	4.8	1.67	16.5
<i>P. fluorescens</i>	9450	12000	21450	4.3	1.75	15.0
Farm yard manure	11760	20000	31760	6.4	2.40	48.5
Neem Seed Powder	8400	12000	20400	4.1	1.45	24.0
Carbofuran	3792	4000	7792	1.6	0.60	15.0
Topsin-M	11124	18000	29124	5.8	2.25	7.5
Bavistin	10548	16000	26548	5.3	1.91	11.5
LSD (0.05)	456.83	728.79	1235.22	0.24	0.15	1.94
LSD (0.01)	622.57	991.81	1683.17	0.32	0.20	2.62

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by *R. solani*

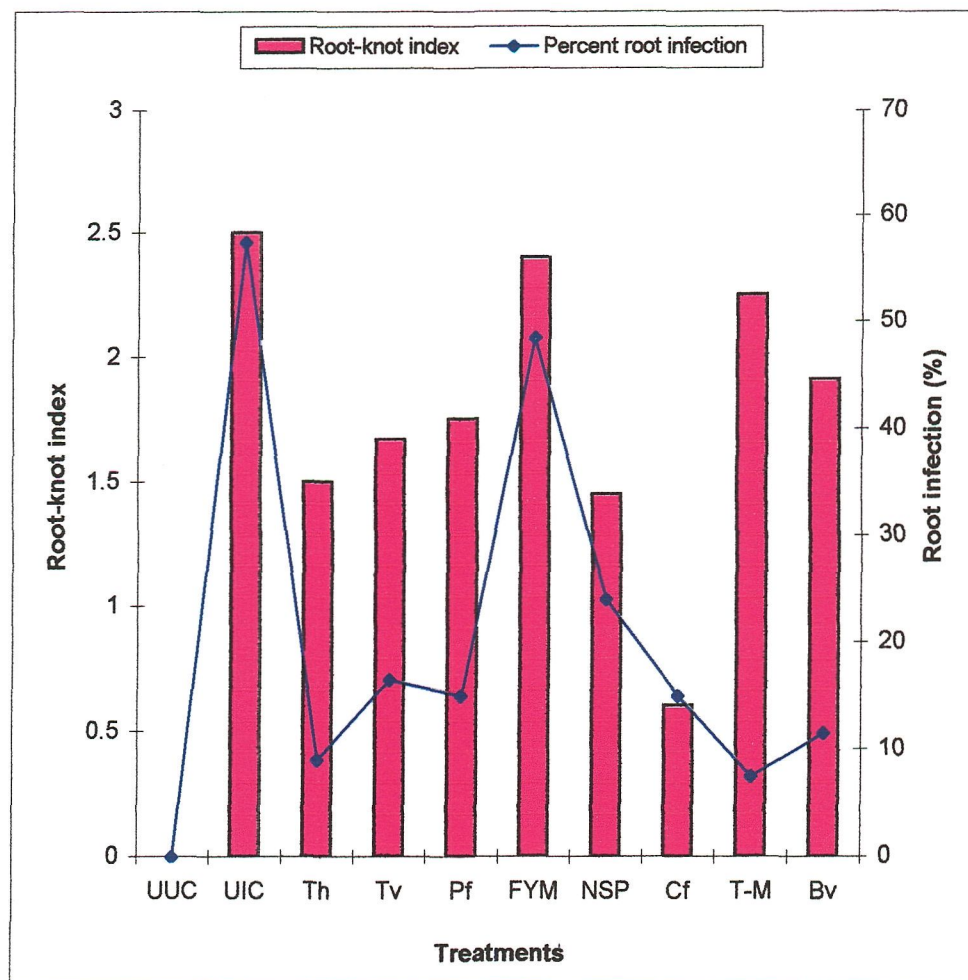


Fig. 15: Comparative efficacy of different additives on disease development in tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

Table 25:

Comparative efficacy of biocontrol agents, organic additives and pesticides on plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil), *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Number of fruits	Fruit weight (g)	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
			Shoot height	Root length	Total	Shoot	Root	Total	Shoot	Root	Total
Untreated-uninoculated Control	10.6	222.5	45.1	28.0	73.1	173.3	37.0	210.3	36.7	10.5	47.4
Untreated inoculated control	2.2 (79.2) ^b	40.0 (82.0)	12.5 (72.3)	6.0 (78.6)	18.5 (74.6)	43.1 (75.1)	8.0 (78.4)	51.1 (75.7)	8.3 (77.4)	2.0 (80.9)	10.4 (78.1)
<i>T. harzianum</i>	9.0 (15.1)	185.0 (16.9)	38.9 (13.7)	23.1 (17.5)	62.0 (15.2)	148.0 (14.6)	30.7 (17.0)	178.7 (15.0)	30.6 (16.6)	8.5 (19.0)	39.1 (17.5)
<i>T. virens</i>	8.6 (18.9)	175.0 (21.3)	37.7 (16.4)	22.0 (21.4)	59.7 (18.3)	140.9 (18.7)	29.0 (21.6)	169.9 (19.2)	29.1 (20.7)	8.0 (23.8)	37.1 (21.7)
<i>P. fluorescens</i>	8.8 (17.0)	180.0 (19.1)	38.0 (15.7)	22.3 (20.4)	60.3 (17.5)	143.0 (17.5)	29.4 (20.5)	172.4 (18.0)	29.5 (19.6)	8.1 (22.9)	37.6 (20.7)
Farm yard manure	4.6 (56.6)	90.0 (59.6)	19.3 (57.2)	10.5 (62.5)	29.8 (59.2)	72.8 (58.0)	14.3 (61.4)	87.1 (58.6)	14.6 (60.2)	3.8 (63.8)	18.4 (61.2)
Neem Seed Powder	8.0 (24.5)	162.5 (27.0)	36.7 (18.6)	21.5 (23.2)	58.2 (20.4)	133.5 (23.0)	27.3 (26.2)	160.8 (23.5)	27.4 (25.3)	7.5 (28.6)	34.9 (26.4)
Carbofuran	8.4 (20.8)	170.0 (23.6)	38.3 (15.1)	22.7 (18.9)	61.0 (16.6)	144.5 (16.6)	29.7 (19.7)	174.2 (17.2)	29.8 (18.8)	8.2 (21.9)	38.0 (19.8)
Topsin-M	7.8 (26.4)	157.5 (29.2)	35.9 (20.4)	20.9 (25.4)	56.8 (22.3)	131.9 (23.9)	27.0 (27.0)	158.9 (24.4)	27.1 (26.2)	7.4 (29.5)	34.5 (27.2)
Bavistin	8.2 (22.6)	167.5 (24.7)	37.1 (17.7)	21.7 (22.5)	58.8 (19.6)	140.0 (19.2)	28.7 (22.4)	168.7 (19.8)	29.0 (21.0)	7.9 (24.8)	36.9 (22.2)
LSD (0.05)	0.32	9.97	1.73	1.13	3.17	7.45	1.33	10.50	1.51	0.42	2.40
LSD (0.01)	0.43	13.44	2.34	1.52	4.27	10.06	1.79	14.16	2.04	0.57	3.24

^aEach value is an average of five replicates

^bFigures in parentheses are percent reduction over untreated uninoculated control

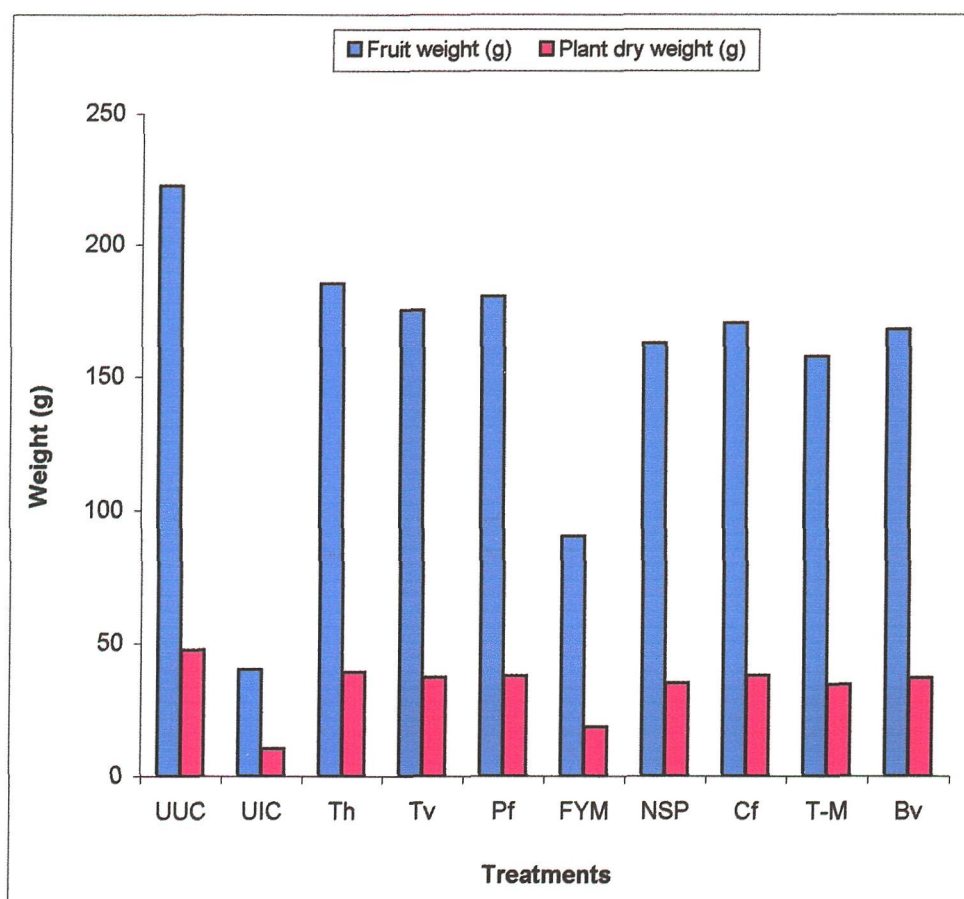


Fig. 16: Comparative efficacy of different additives on plant growth and fruit yield of tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

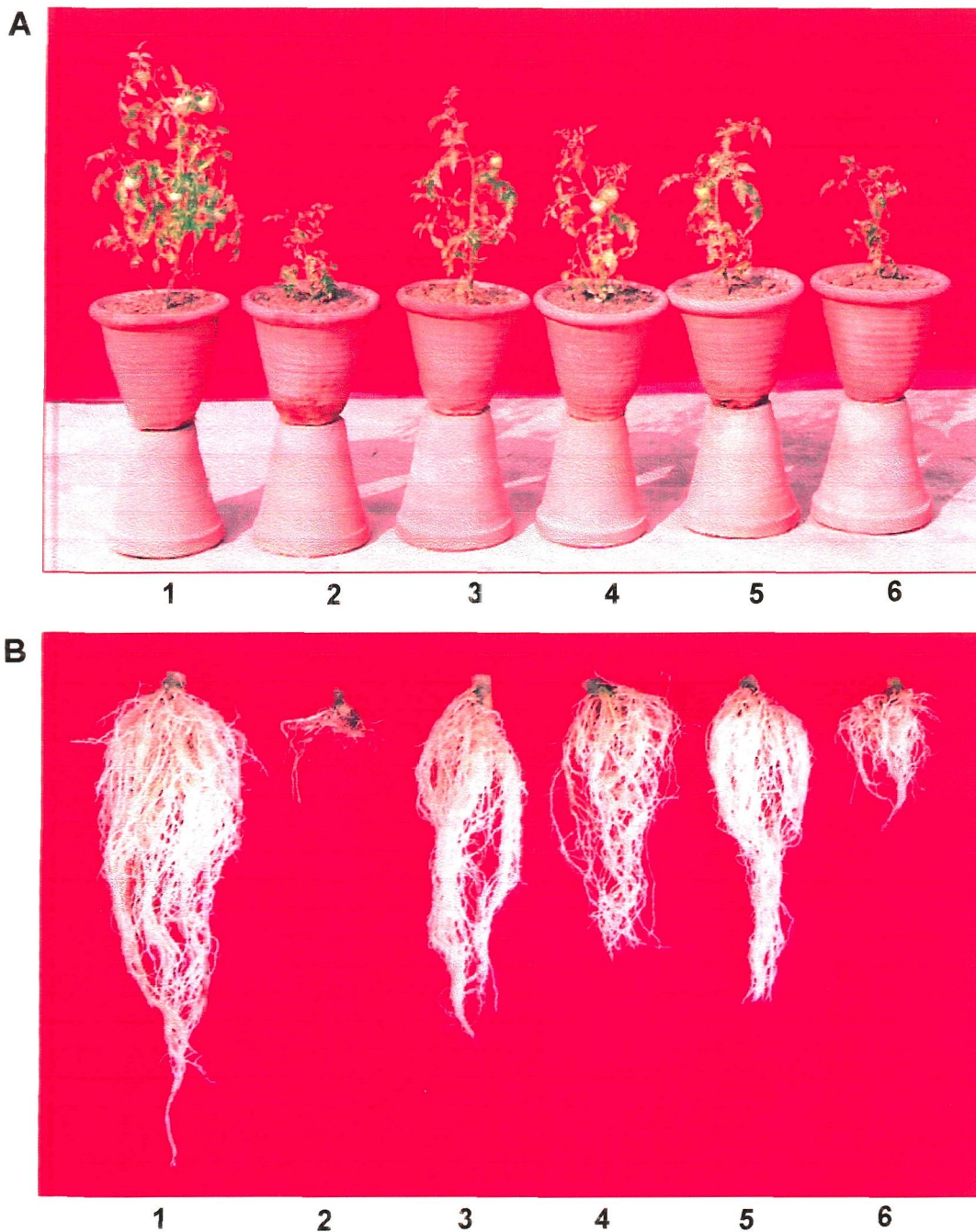


Plate 18 (i): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 3= *T. harzianum* @ 250 mg/5 kg soil

4= *T. virens* @ 250 mg/5 kg soil
 5= *P. fluorescens* @ 250 mg/5 kg soil
 6= FYM @ 7500 mg/5 kg soil

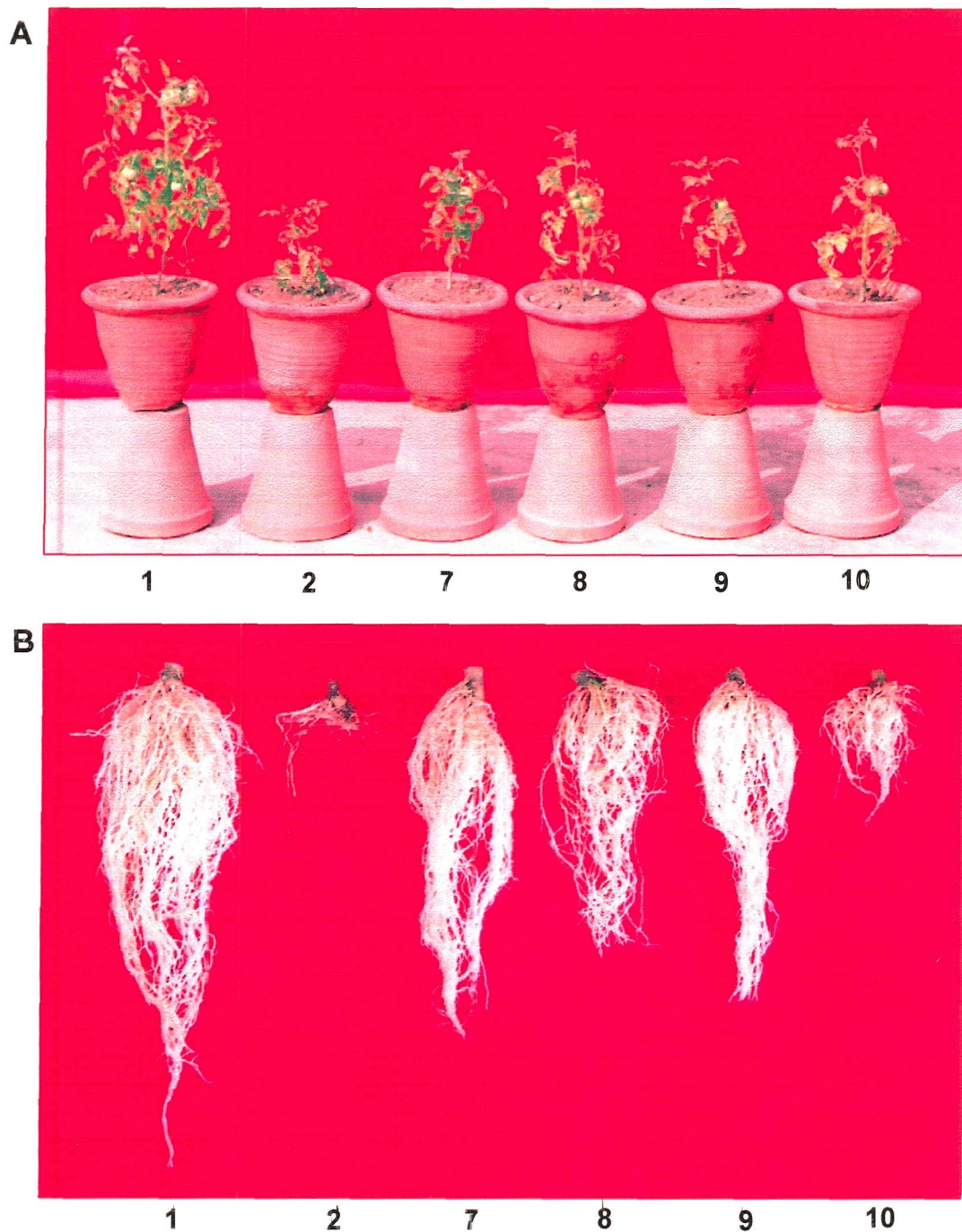


Plate 18 (ii): Comparative efficacy of different additives on the aerial growth (A) and disease development on roots of tomato cv. K-25 (B) inoculated with *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) simultaneously

1= Uninoculated untreated
 2= Inoculated untreated
 7= NSP @ 1250 mg/5 kg soil

8= Carbofuran @ 167 mg/5 kg soil
 9= Topsin-M @ 12 mg/5 kg soil
 10= Bavistin @ 10 mg/5 kg soil

carbofuran, *P. fluorescens*, *T. virens*, neem seed powder and farmyard manure, respectively [Table 24; Fig. 15; Plate 17 (i), (ii)]. The highest Rf (6.9), RKI (2.50) and PRI (57.5) was recorded in untreated inoculated plants.

5.7 *M. incognita*, *F. oxysporum* and *R. solani* simultaneously

Data presented in (Table 25) regarding the comparative efficacy of different additives against *M. incognita*, *F. oxysporum* and *R. solani* simultaneously inoculated plants of tomato cv. K-25 indicated that in general, application of all the treatments were able to increase the plant growth and fruit yield as compared to untreated inoculated plants [Fig. 16; Plate 18 (i), (ii)].

Analyses of the data indicated that the differences on the effect of different treatments on plant dry weight and fruit yield was mostly significant ($P \leq 0.05$). However, highest improvement in plant dry weight (39.1 g) and fruit yield (185.0 g) was found in plants treated with *T. harzianum* followed by *P. fluorescens*, *T. virens*, carbofuran, bavistin, neem seed powder, topsin-M and farmyard manure, respectively as compared to untreated inoculated plants.

All the treatments were also able to reduce the root-knot development, *M. incognita* reproduction rate and percent root infection by *F. oxysporum* and *R. solani*. The highest reduction in nematode Rf (1.0) and RKI (0.50) was found in carbofuran treated plants followed by *T. harzianum*, *P. fluorescens*, *T. virens*, neem seed powder, bavistin, topsin-M and farmyard manure, respectively. The highest reduction in root infection due to both the fungi (10.0%) was found in plants treated with bavistin followed by topsin-M, *T. harzianum*, *P. fluorescens*, *T. virens*, carbofuran, neem seed powder and farmyard manure, respectively [Table 26; Fig. 17; Plate 18 (i), (ii)]. The highest Rf (3.4), RKI (1.50) and PRI (78.5%) was found in untreated inoculated plants.

6. Compatibility test, *in-vitro*, among fungal and bacterial biocontrol agents and thereof with pesticides and organic amendments

Data regarding the compatibility of biocontrol agents, organic amendments and pesticides (Table 27) indicated that *T. harzianum* isolate TH-H-3 and *T. virens* TV-K-3 were 100% compatible with both the organic amendments viz., farmyard manure and neem seed powder. In case of pesticides, both the fungal biocontrol agents were 100%

Table 26: Comparative efficacy of different biocontrol agents, organic additives and pesticides on nematode multiplication, root-knot index and percent root infection by fungal pathogens in tomato cv. K-25 simultaneously inoculated with *Meloidogyne incognita* (5000 J₂/5 kg soil), *Fusarium oxysporum* (7.5x10⁶ cfu/5 kg soil) and *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions^a

Treatments	Final nematode population			Reproduction factor	^b Root-knot index	^c Percent root infection
	Root (Total)	Soil (5 Kg)	Total			
Untreated-uninoculated control	--	--	--	--	--	--
Untreated-inoculated control	3040	14000	17040	3.4	1.50	78.5
<i>T. harzianum</i>	6140	4000	10140	2.0	1.00	13.5
<i>T. virens</i>	7056	4000	11056	2.2	1.20	21.5
<i>P. fluorescens</i>	6380	4000	10960	2.2	1.10	17.5
Farm yard manure	5148	12000	17148	3.4	1.50	70.0
Neem Seed Powder	6552	4000	10552	2.1	1.00	25.0
Carbofuran	2970	2000	4970	1.0	0.50	23.5
Topsin-M	8640	8000	16640	3.3	1.35	12.5
Bavistin	8036	6000	14036	2.8	1.25	10.0
LSD (0.05)	271.81	295.75	640.77	0.18	0.11	1.44
LSD (0.01)	371.34	402.23	873.12	0.25	0.15	1.94

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

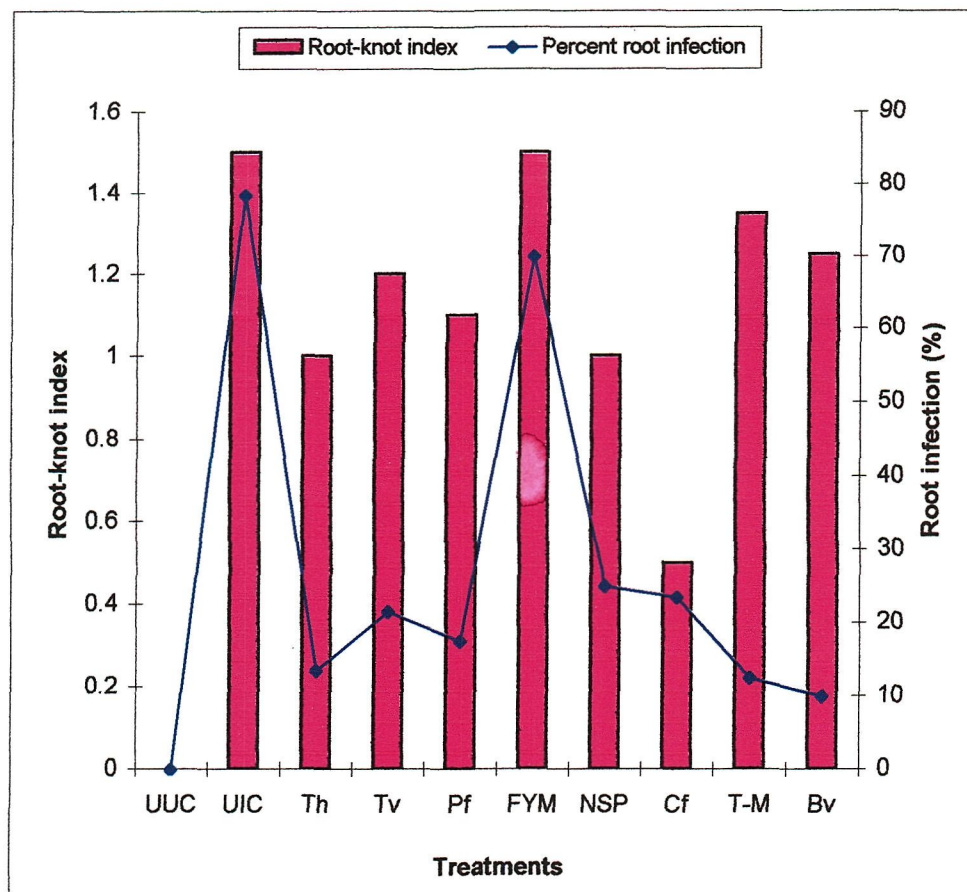


Fig. 17: Comparative efficacy of diferent additives on disease development in tomato cv. K-25 simultaneously inoculated with *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5x10⁶ cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) under pot conditions

UUC = Untreated uninoculated control
 UIC = Untreated inoculated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure
 NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin

Table 27 (i): Compatibility of *Trichoderma harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 and *Pseudomonas fluorescens* isolate PS-4 with organic amendments and pesticides under *in vitro* conditions

Treatments	Growth of biocontrol agents (cm)		
	<i>T. harzianum</i> (TH-H-3)	<i>T. virens</i> (TV-K-3)	<i>P. fluorescens</i> (PS-4)
Farmyard manure	9.0 (+100.0)	9.0 (+100.0)	9.0 (+100.0)
Neem seed powder	9.0 (+100.0)	9.0 (+100.0)	9.0 (+100.0)
Carbofuran	9.0 (+100.0)	9.0 (+100.0)	9.0 (+100.0)
Topsin-M	0.0 (-100.0)	0.0 (-100.0)	9.0 (+100.0)
Bavistin	0.0 (-100.0)	0.0 (-100.0)	9.0 (+100.0)
Control	9.0	9.0	9.0

^aEach value is an average of five replicates

^bFigures in parentheses are percent compatibility (+ compatible, - incompatible)

Table 27 (ii): Compatibility of *Trichoderma harzianum* isolate TH-H-3 and *T. virens* isolate TV-K-3 with *Pseudomonas fluorescens* isolate PS-4 under *in vitro* conditions

Treatments	Growth of biocontrol agents (cm)	
	<i>T. harzianum</i> (TH-H-3)	<i>T. virens</i> (TV-K-3)
<i>Pseudomonas fluorescens</i> (PS-4)	9.0 (100.0)	7.0 (77.8)

^aEach value is an average of five replicates

^bFigures in parentheses are percent compatibility

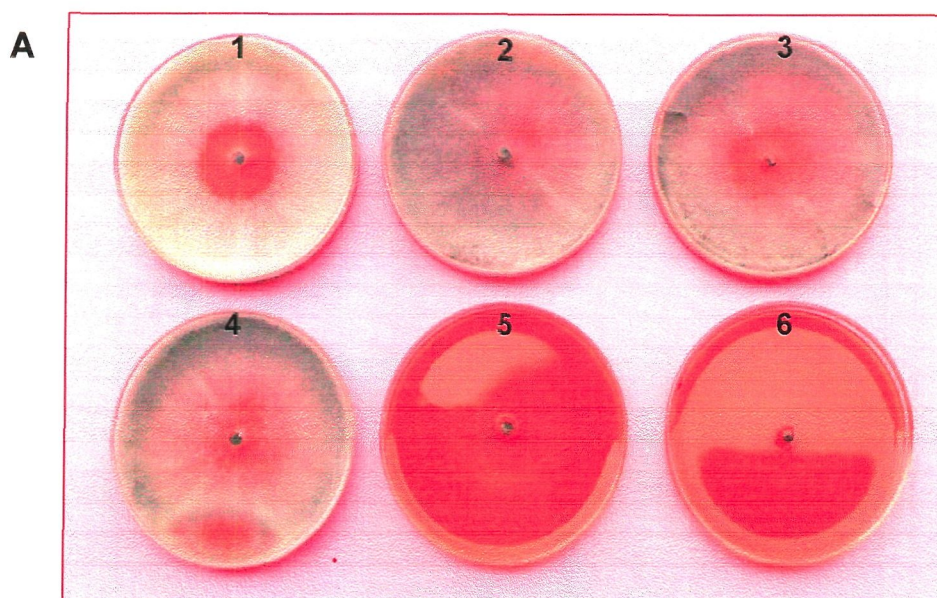


Plate 19 (i): Petriplates showing compatibility of *T. harzianum* isolate TH-H-3 (A) and *T. virens* isolate TV-K-3 (B) with organic amendments and pesticides

1= Untreated control
2= Farmyard manure
3= Neem seed powder

4= Carbofuran
5= Topsin-M
6= Bavistin

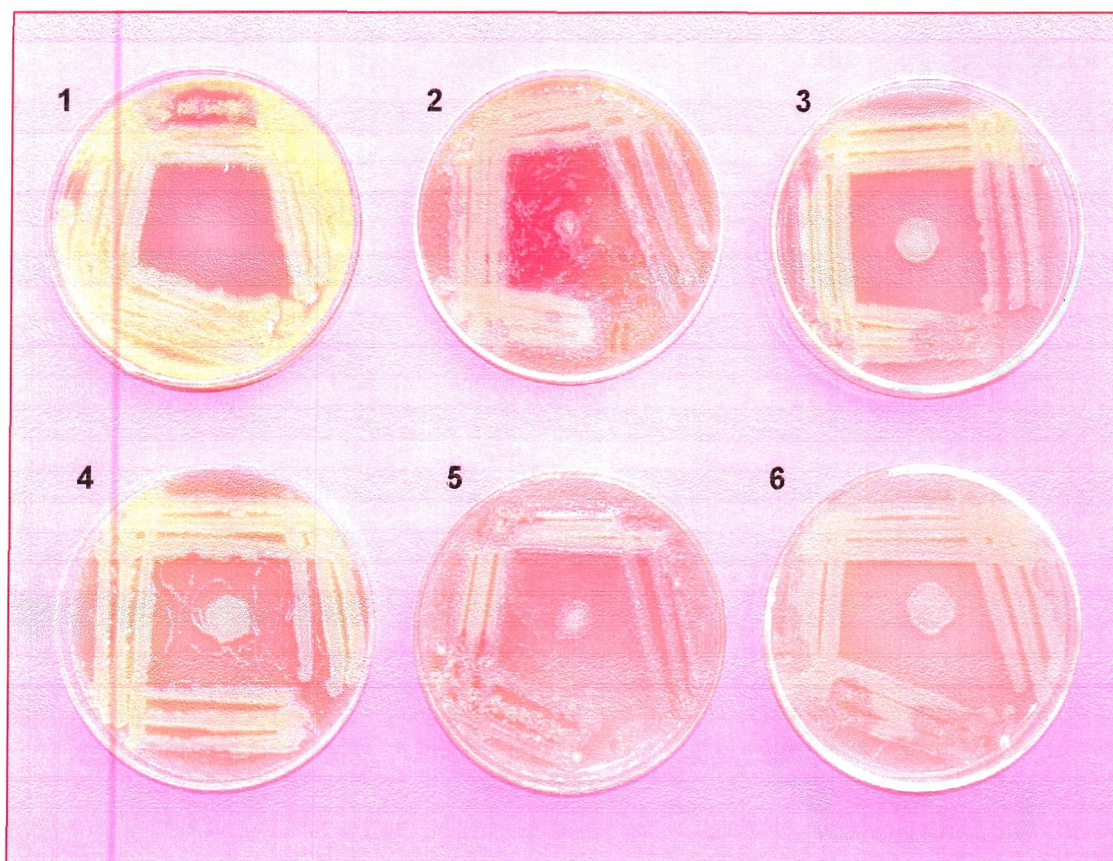


Plate 19 (ii): Petriplates showing compatibility of *P. fluorescens* isolate PS-4 with organic amendments and pesticides

1= Untreated control
2= Farmyard manure
3= Neem seed powder

4= Carbofuran
5= Topsin-M
6= Bavistin

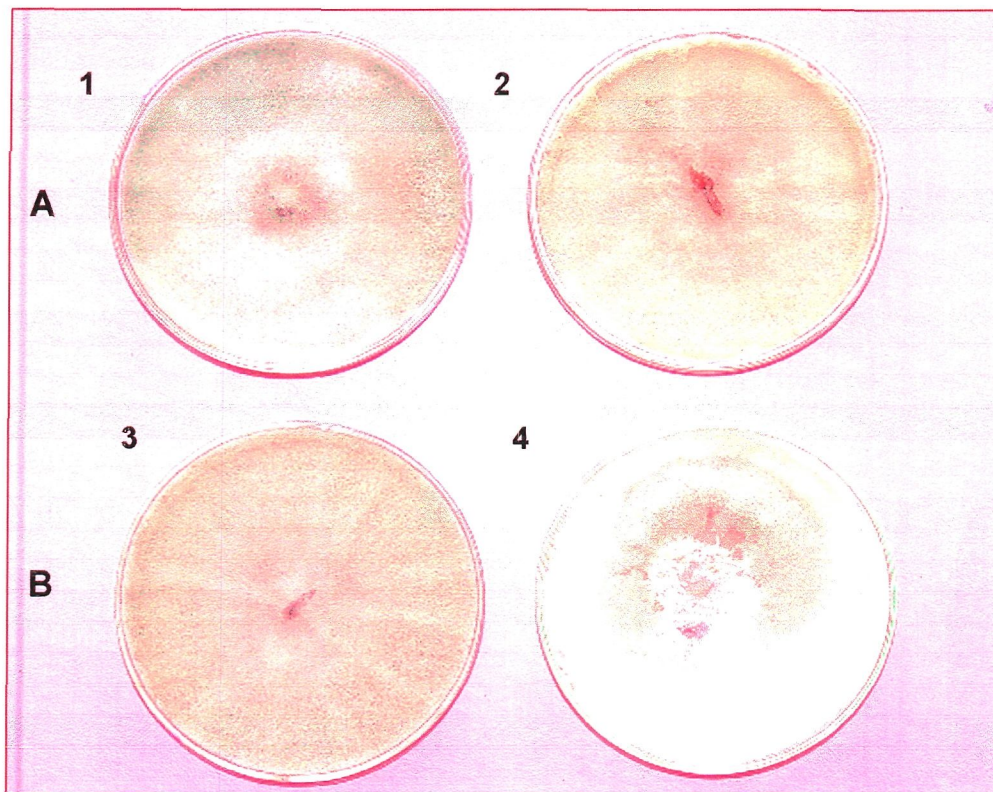


Plate 19 (iii): Petriplates showing compatibility of *T. harzianum* isolate TH-H-3 (A) and *T. virens* isolate TV-K-3 (B) with *P. fluorescens* isolate PS-4

(A) 1= *T. harzianum* isolate TH-H-3 2= *T. harzianum* + *P. fluorescens*

(B) 3= *T. virens* isolate TV-K-3 4= *T. virens* + *P. fluorescens*

compatible with carbofuran, while they were incompatible with topsin-M and bavistin [Table 27 (i); Plate 19 (i)]. However, *P. fluorescens* isolate PS-4 was found 100% compatible with all the organic amendments and pesticides [Table 27 (i); Plate 19 (ii)]. Among biocontrol agents, *T. harzianum* (TH-H-3) and *T. virens* (TV-K-3) isolates exhibited 100% and 77.8% compatibility, respectively with *P. fluorescens* (PS-4) [Table 27 (ii); Plate 19 (iii)].

7. Efficacy of biocontrol agents, organic amendments and pesticides alone and in combination, against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in nursery under sick plot conditions

7.1 Comparative efficacy of various treatments alone against *M. incognita*, *F. oxysporum* and *R. solani* in nursery bed of tomato cv. K-25

Data presented in [Table 28 (i)] regarding the effect of various treatments used alone on the percent emergence and fresh weight of seedling, grown in sick field infested with *M. incognita*, *F. oxysporum* and *R. solani*, indicated that all the treatments applied alone were found to be effective ($P \leq 0.05$) in increasing the percent emergence and fresh weight of seedling and also in reducing the disease severity as compared to untreated control (Plate 20 A). All the treatments not only able to enhance the percent emergence of the seedling but also increase the fresh weight of the seedling. Analyses of data indicated that differences in the emergence and fresh weight of the seedlings among different treatments were mostly significant ($P \leq 0.05$). However, bavistin was found highly effective in increasing the percent emergence (48.6) and fresh weight (132.5 g) of the seedlings followed by *T. harzianum*, carbofuran, *P. fluorescens*, *T. virens*, neem seed powder, topsin-M and farmyard manure, respectively.

Treatment with bavistin was found most effective in reducing the PRI (0.5) followed by *T. harzianum*, carbofuran, *P. fluorescens*, *T. virens*, neem seed powder, topsin-M and farmyard manure, respectively. However, treatment with carbofuran was found most effective in reducing RKI (0.5) followed by neem seed powder, bavistin, *T. harzianum*, *P. fluorescens*, *T. virens*, topsin-M and farmyard manure, respectively.

7.2 Comparative efficacy of various treatments combinations of *T. harzianum* with *P. fluorescens*, neem seed powder, farmyard manure and carbofuran against *M. incognita*, *F. oxysporum* and *R. solani* in nursery bed of tomato cv. K-25

Studies regarding the effect of *T. harzianum* in combination with various other additives on the percent emergence and fresh weight of the seedlings grown in sick field infested with *M. incognita*, *F. oxysporum* and *R. solani*, indicated that all the treatment combinations were highly effective ($P \leq 0.01$) in increasing the percent emergence and fresh weight of the seedlings and also reducing disease severity as compared to untreated control [Table 28 (ii); Plate 20].

Analyses of data indicated that differences in percent emergence and fresh weight of seedlings were mostly significant ($P \leq 0.05$) among the different treatments. However, combined treatment with *T. harzianum* + farmyard manure + neem seed powder + carbofuran (all the additives were applied as one fourth doses of standard dose) was found most effective in increasing the percent emergence (71.4) and fresh weight (218.7 g) of the seedlings followed by *T. harzianum* + neem seed powder + carbofuran (one fourth doses), *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *T. harzianum* + *P. fluorescens* + neem seed powder (one fourth doses), *T. harzianum* + farmyard manure + carbofuran (one third doses), *T. harzianum* + farmyard manure + neem seed powder (one third doses), *T. harzianum* + *P. fluorescens* + farmyard manure (one third doses), *T. harzianum* + carbofuran (half doses), *T. harzianum* + neem seed powder (half doses), *T. harzianum* + *P. fluorescens* (half doses), and *T. harzianum* + farmyard manure (half doses), respectively. Highest reduction in RKI (0.0) and PRI (7.5) was also found in nursery beds treated with *T. harzianum* + farmyard manure + neem seed powder + carbofuran (one fourth doses).

7.3 Comparative efficacy of various treatments combinations of *T. virens* with *P. fluorescens*, neem seed powder, farmyard manure and carbofuran against *M. incognita*, *F. oxysporum* and *R. solani* in nursery bed of tomato cv. K-25

Data [Table 28 (iii)] regarding the management of *M. incognita*, *F. oxysporum* and *R. solani* on the percent emergence and fresh weight of the seedlings by the application of *T. virens* in combinations with various other additives indicated that all the

Table 28 (i): Comparative efficacy of various treatments alone on percent seed germination, seedlings growth and disease development on tomato cv. K-25 in nursery beds infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*^a

Treatments	Average emergence of seedlings/bed	Average seedling fresh weight/bed	^b Root-knot index	^c Percent root infection
Untreated control	25.0	60.3	3.5	75.0
<i>T. harzianum</i>	48.0 (92.0) ^d	130.0 (115.6)	1.7	15.0
<i>T. virens</i>	43.8 (75.2)	118.0 (95.7)	2.1	18.5
<i>P. fluorescens</i>	45.0 (80.0)	121.7 (101.8)	2.0	17.5
Farm yard manure	30.0 (25.0)	83.7 (38.8)	3.1	67.5
Neem seed powder	43.4 (73.6)	115.7 (91.9)	1.3	20.0
Carbofuran	46.8 (87.2)	128.3 (112.8)	0.5	25.0
Topsin-M	40.2 (60.8)	104.7 (73.6)	2.9	15.5
Bavistin	48.6 (94.4)	132.5 (119.7)	1.7	11.5
LSD (0.05)	4.56	11.35	0.21	3.23
LSD (0.01)	6.20	15.42	0.29	4.39

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

^dFigures in parentheses are percent increase over untreated control

treatments not only suppressed the root infection but also increased the percent emergence of the seedlings and their fresh weight (Plate 20 A).

Analyses of data indicated that differences in the percent emergence of the seedlings among different treatments were mostly significant ($P \leq 0.05$). However, combined treatment with *T. virens* + farmyard manure + neem seed powder + carbofuran (one fourth doses) was most effective in increasing the percent emergence (67.4) and fresh weight (204.0 g) of the seedlings followed by *T. virens* + neem seed powder + carbofuran (one fourth doses), *T. virens* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *T. virens* + *P. fluorescens* + neem seed powder (one third doses), *T. virens* + farmyard manure + carbofuran (one third doses), *T. virens* + farmyard manure + neem seed powder (one third doses), *T. virens* + *P. fluorescens* + farmyard manure (one third doses), *T. virens* + carbofuran (half doses), *T. virens* + neem seed powder (half doses), *T. virens* + *P. fluorescens* (half doses), and *T. virens* + farmyard manure (half doses), respectively. Highest reduction in RKI (0.1) and PRI (11.5%) was also found in nursery beds treated with *T. virens* + farmyard manure + neem seed powder + carbofuran (one fourth doses).

7.4 Comparative efficacy of various treatments combinations of *P. fluorescens* with *T. harzianum*, *T. virens* neem seed powder, farmyard manure, carbofuran, topsin-M and bavistin against *M. incognita*, *F. oxysporum* and *R. solani* in nursery bed of tomato cv. K-25

Studies regarding the effect of *P. fluorescens* in combination with various other additives on the percent emergence and fresh weight of the seedlings grown in sick beds indicated that all the treatment combinations were highly effective ($P \leq 0.01$) in increasing the percent emergence and fresh weight of the seedlings as compared to untreated control [Table 28 (iv); Plate 20].

Analyses of data indicated that differences in percent emergence of seedlings among different treatments were mostly significant ($P \leq 0.05$). However, highest percent emergence (69.0) and fresh weight (211.3 g) of the seedlings was found in nursery beds treated with *P. fluorescens* + farmyard manure + neem seed powder + carbofuran (one fourth doses) followed by *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses), *P. fluorescens* + *T. harzianum* + farmyard manure + neem

Table 28 (ii): Comparative efficacy of various treatment combinations of *Trichoderma harzianum* with *Pseudomonas fluorescens*, neem seed powder, farmyard manure and carbofuran on percent seed germination, seedlings growth and disease development on tomato cv. K-25 in nursery beds infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*^a

Treatments	Average emergence of seedlings /bed	Average seedling fresh weight/bed	^b Root-knot index	^c Percent root infection
Untreated control	25.0	60.3	3.5	75.0
<i>T. harzianum</i> + <i>P. fluorescens</i>	58.4 (133.6)	161.0 (167.0)	0.9	18.5
<i>T. harzianum</i> + FYM	51.8 (107.2)	139.0 (130.5)	1.5	19.0
<i>T. harzianum</i> + NSP	58.6 (134.4)	163.3 (170.8)	0.7	15.5
<i>T. harzianum</i> + Carbofuran	60.2 (140.8)	172.0 (185.2)	0.3	15.0
<i>T. harzianum</i> + <i>P. fluorescens</i> + FYM	63.4 (153.6)	185.7 (208.0)	0.9	17.5
<i>T. harzianum</i> + <i>P. fluorescens</i> + NSP	66.4 (165.6)	198.3 (228.9)	0.3	11.5
<i>T. harzianum</i> + FYM + NSP	64.2 (156.8)	188.3 (212.3)	0.7	18.5
<i>T. harzianum</i> + FYM + Carbofuran	65.0 (160.0)	193.3 (220.6)	0.3	13.5
<i>T. harzianum</i> + NSP + Carbofuran	68.8 (175.2)	208.3 (245.4)	0.1	10.0
<i>T. harzianum</i> + FYM + NSP + Carbofuran	71.4 (185.6)	218.7 (262.7)	0.0	7.5
<i>T. harzianum</i> + <i>P. fluorescens</i> + FYM + NSP	68.0 (172.0)	207.3 (243.8)	0.2	10.0
LSD (0.05)	6.04	16.21	0.08	1.85
LSD (0.01)	8.19	21.90	0.11	2.52

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

^dFigures in parentheses are percent increase over untreated control

Table 28 (iii):

Comparative efficacy of various treatment combinations of *Trichoderma virens* with *Pseudomonas fluorescens*, neem seed powder, farmyard manure and carbofuran on percent seed germination, seedlings growth and disease development on tomato cv. K-25 in nursery beds infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*^a

Treatments	Average emergence of seedlings/bed	Average seedling fresh weight/bed	^b Root-knot index	^c Percent root infection
Untreated control	25.0	60.3	3.5	75.0
Tv + Pf	56.8 (127.2)	153.7 (154.9)	1.3	19.5
Tv + FYM	46.8 (87.2)	125.3 (107.8)	1.9	23.0
Tv + NSP	57.4 (129.6)	156.0 (158.7)	0.9	20.5
Tv + CF	59.2 (136.8)	166.0 (175.3)	0.7	21.5
Tv + Pf + FYM	60.0 (140.0)	172.0 (185.2)	1.2	17.5
Tv + Pf + NSP	64.6 (158.4)	190.0 (215.1)	0.5	13.5
Tv + FYM + NSP	60.2 (140.8)	172.7 (186.4)	1.0	19.5
Tv + FYM + CF	63.0 (152.0)	183.3 (204.0)	0.5	17.0
Tv + NSP + CF	66.6 (166.4)	199.7 (231.2)	0.2	13.0
Tv + FYM + NSP + CF	67.4 (169.6)	204.0 (238.3)	0.1	11.5
Tv + Pf + FYM + NSP	65.8 (163.2)	196.3 (225.5)	0.5	12.5
LSD (0.05)	5.91	17.37	0.13	2.73
LSD (0.01)	8.02	23.57	0.18	3.72

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

^dFigures in parentheses are percent increase over untreated control

seed powder (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + topsin-M (one fourth doses), *P. fluorescens* + neem seed powder + carbofuran (one third doses), *P. fluorescens* + neem seed powder + bavistin (one third doses), *P. fluorescens* + *T. harzianum* + neem seed powder (one third doses), *P. fluorescens* + *T. virens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + neem seed powder + topsin-M (one third doses), *P. fluorescens* + *T. virens* + neem seed powder (one third doses), *P. fluorescens* + farmyard manure + carbofuran (one third doses), *P. fluorescens* + *T. harzianum* + farmyard manure (one third doses), *P. fluorescens* + farmyard manure + bavistin (one third doses), *P. fluorescens* + farmyard manure + topsin-M (one third doses), *P. fluorescens* + farmyard manure + neem seed powder (one third doses), *P. fluorescens* + bavistin (half doses), *P. fluorescens* + *T. virens* + farmyard manure (one third doses), *P. fluorescens* + carbofuran (half doses), *P. fluorescens* + *T. harzianum* (half doses), *P. fluorescens* + topsin-M (half doses), *P. fluorescens* + neem seed powder (half doses), *P. fluorescens* + *T. virens* (half doses) and *P. fluorescens* + farmyard manure (half doses), respectively. Highest reduction in RKI (0.1) was found in seedlings grown in nursery beds treated with *P. fluorescens* + farmyard manure + neem seed powder + carbofuran (one fourth doses). However, highest reduction in PRI (5.0) was found in nursery beds treated with *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses).

8. Evaluation of various treatments of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in sick field conditions

Studies regarding the effect of package of selected treatments on the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 under sick field conditions indicated that in general, all the treatments were highly effective ($P \leq 0.01$) in improving the plant growth and fruit yield and suppressing nematode and fungal infection as compared to untreated control (Table 29; Fig. 18, 19; Plate 21).

The highest increase in plant dry weight (89.8%) and fruit yield (92.7%) was found in plots treated with *T. harzianum* + farmyard manure + neem seed powder + carbofuran (one fourth doses) followed by the *P. fluorescens* + farmyard manure + neem

Table 28 (iv): Comparative efficacy of various treatment combinations of *Pseudomonas fluorescens* with *Trichoderma harzianum*, *T. virens*, neem seed powder, farmyard manure and carbofuran on percent seed germination, seedlings growth and disease development on tomato cv. K-25 in nursery beds infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*^a

Treatments	Average emergence of seedlings/bed	Average seedling fresh weight/bed	^b Root-knot index	^c Percent root infection
Untreated control	25.0	60.3	3.5	75.0
Pf + Th	58.4 (133.6)	161.0 (167.0)	0.9	18.5
Pf + Tv	56.8 (127.2)	153.7 (154.9)	1.3	19.5
Pf + FYM	50.0 (100.0)	135.7 (125.0)	1.7	21.5
Pf + NSP	57.6 (130.4)	158.3 (162.5)	0.7	19.5
Pf + CF	60.0 (140.0)	169.7 (181.4)	0.5	18.0
Pf + TM	58.0 (132.0)	161.5 (167.8)	1.5	13.5
Pf + B	60.6 (142.4)	173.0 (186.9)	1.2	9.0
Pf + Th + FYM	63.4 (153.6)	185.7 (208.0)	0.9	17.5
Pf + Th + NSP	66.4 (165.6)	198.3 (228.9)	0.3	11.5
Pf + FYM + NSP	61.2 (144.8)	176.0 (191.9)	0.9	17.5
Pf + FYM + CF	64.0 (156.0)	186.7 (209.6)	0.3	15.5

Pf + NSP + CF	67.0 (168.0)	202.0 (235.0)	0.1	12.5
Pf + Tv + FYM	60.0 (140.0)	172.0 (185.2)	1.2	17.5
Pf + Tv + NSP	64.6 (158.4)	190.0 (215.1)	0.5	13.5
Pf + FYM + TM	62.4 (149.6)	180.0 (198.5)	1.4	13.0
Pf + NSP + TM	65.8 (163.2)	195.5 (224.2)	0.6	7.5
Pf + FYM + B	63.2 (152.8)	183.9 (205.0)	1.0	8.0
Pf + NSP + B	66.8 (167.2)	200.0 (231.7)	0.3	6.5
Pf + Th + FYM + NSP	68.0 (172.0)	207.3 (243.8)	0.2	10.0
Pf + Tv + FYM + NSP	65.8 (163.2)	196.3 (225.5)	0.5	12.5
Pf + FYM + NSP + CF	69.0 (176.0)	211.3 (250.4)	0.1	9.5
Pf + FYM + NSP + TM	67.0 (168.0)	202.7 (236.2)	0.5	7.0
Pf + FYM + NSP + B	68.8 (175.2)	210.7 (249.4)	0.2	5.0
LSD (0.05)	6.25	18.73	0.08	1.96
LSD (0.01)	8.48	25.20	0.11	2.66

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

^dFigures in parentheses are percent increase over untreated control

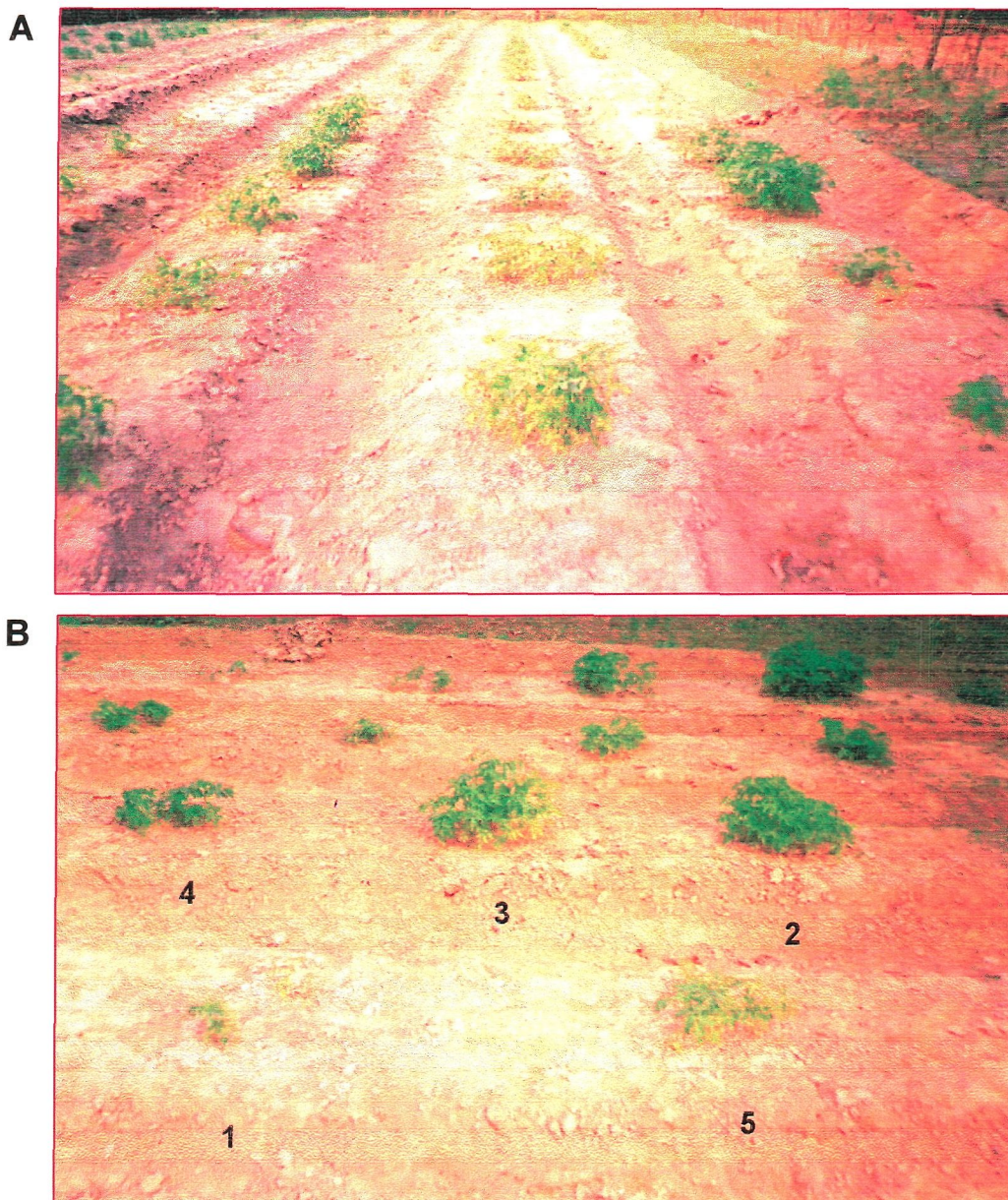


Plate 20: Comparative efficacy of various treatments alone and in combinations on the germination and seedlings growth of tomato cv. K-25 in nursery beds infested with *M. incognita*, *F. oxysporum* and *R. solani*

- (A) A view of the nursery beds infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*
- (B) A view of seedlings grown in nursery beds treated with selected combinations of various treatment materials along with seedlings grown in untreated beds

1= Untreated control

2= Th + FYM + NSP + Cf

4= Th + Pf + FYM + NSP

5= Pf + FYM + NSP + B

Table 29: Comparative efficacy of selected treatment combinations on disease development, nematode multiplication, plant growth and fruit yield of tomato cv. K- 25 grown in a field infested with *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*^a

Treatments	Fruit yield (kg/plot)	Plant fresh weight (kg/plot)	Plant dry weight (kg/plot)	Final Nematode Population			Reproduction factor	Root-knot index	Percent root infection
				Root (per g)	Soil (250 g)	Total			
Untreated control	20.00	33.30	7.92	640	5100	5740	2.87	4.00	82.5
Th + Pf + FYM + NSP	35.95 (79.8) ^d	57.06 (71.4)	13.86 (75.0)	190	900	1090	0.55	1.15	15.0
Tv + Pf + FYM + NSP	33.05 (65.25)	53.25 (59.9)	12.91 (63.0)	210	1100	1310	0.66	1.49	25.0
Th + FYM + NSP + Cf	38.54 (92.7)	62.08 (86.4)	15.03 (89.8)	87	600	687	0.34	0.50	13.5
Tv + FYM + NSP + Cf	34.72 (72.6)	55.17 (65.7)	13.33 (68.3)	180	900	1080	0.54	1.25	20.5
Pf + FYM + NSP + Cf	36.38 (81.9)	58.59 (75.9)	14.14 (78.5)	140	800	940	0.47	0.88	17.5
Pf + FYM + NSP + TM	34.20 (71.00)	54.90 (64.9)	13.33 (68.3)	255	1300	1555	0.78	2.00	15.5
Pf + FYM + NSP + B	35.90 (79.5)	57.00 (71.2)	13.79 (74.1)	230	1100	1330	0.67	1.70	10.0
Th + FYM + Cf	32.35 (61.8)	51.30 (54.1)	12.45 (57.2)	170	900	1070	0.54	1.00	21.0
Th + Pf + NSP	33.50 (67.50)	53.95 (62.0)	13.07 (65.0)	200	1000	1200	0.60	1.23	19.0
Th + NSP + Cf	35.43 (77.2)	56.50 (69.7)	13.68 (72.7)	130	800	930	0.47	0.60	18.5
Tv + NSP + Cf	33.50 (67.5)	54.00 (62.2)	13.10 (65.4)	200	900	1100	0.55	1.37	25.5
Pf + NSP + Cf	34.00 (70.0)	54.53 (63.8)	13.27 (67.6)	160	900	1060	0.53	1.00	22.5
Pf + NSP + B	33.87 (69.4)	54.48 (63.6)	13.20 (66.7)	250	1200	1450	0.73	1.85	12.5
Pf + NSP + TM	33.0 (65.0)	53.18 (59.7)	12.90 (62.9)	250	1300	1550	0.78	2.15	17.5
L.S.D. (0.05)	2.65	4.26	1.02	18.57	124.16	139.91	0.05	0.09	1.99
L.S.D. (0.01)	3.60	5.79	1.39	25.37	169.91	191.54	0.07	0.13	2.71

^aEach value is an average of five replicates

^bRoot-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

^cPercent root infection by fungal pathogens

^dFigures in parentheses are percent reduction over untreated control

Pi of *Meloidogyne incognita* = 2000 I₂/250 g soil

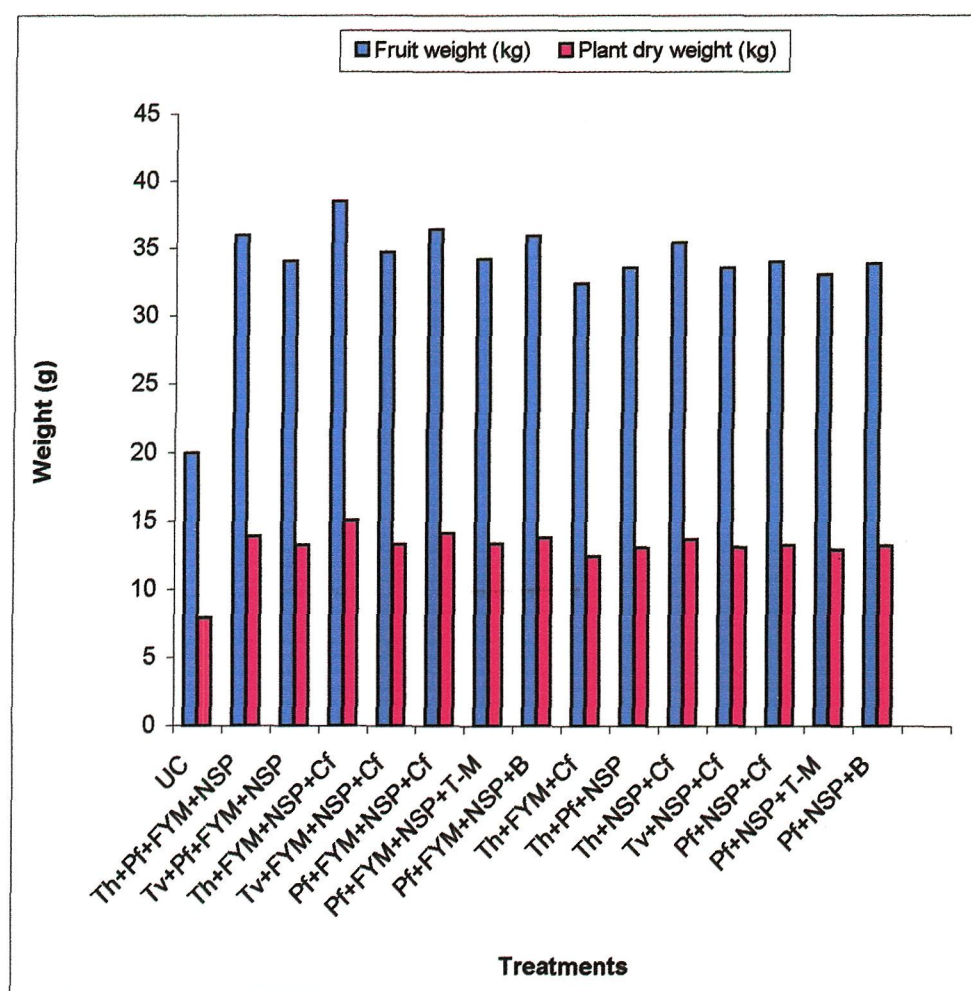


Fig. 18: Comparative efficacy of selected treatments on plant growth and fruit yield of tomato cv. K-25 grown in sick field

UC = Untreated control

Th = *Trichoderma harzianum*

Tv = *T. virens*

Pf = *Pseudomonas fluorescens*

FYM = Farmyard manure

NSP = Neem seed powder

Cf = Carbofuran

T-M = Topsin-M

Bv = Bavistin

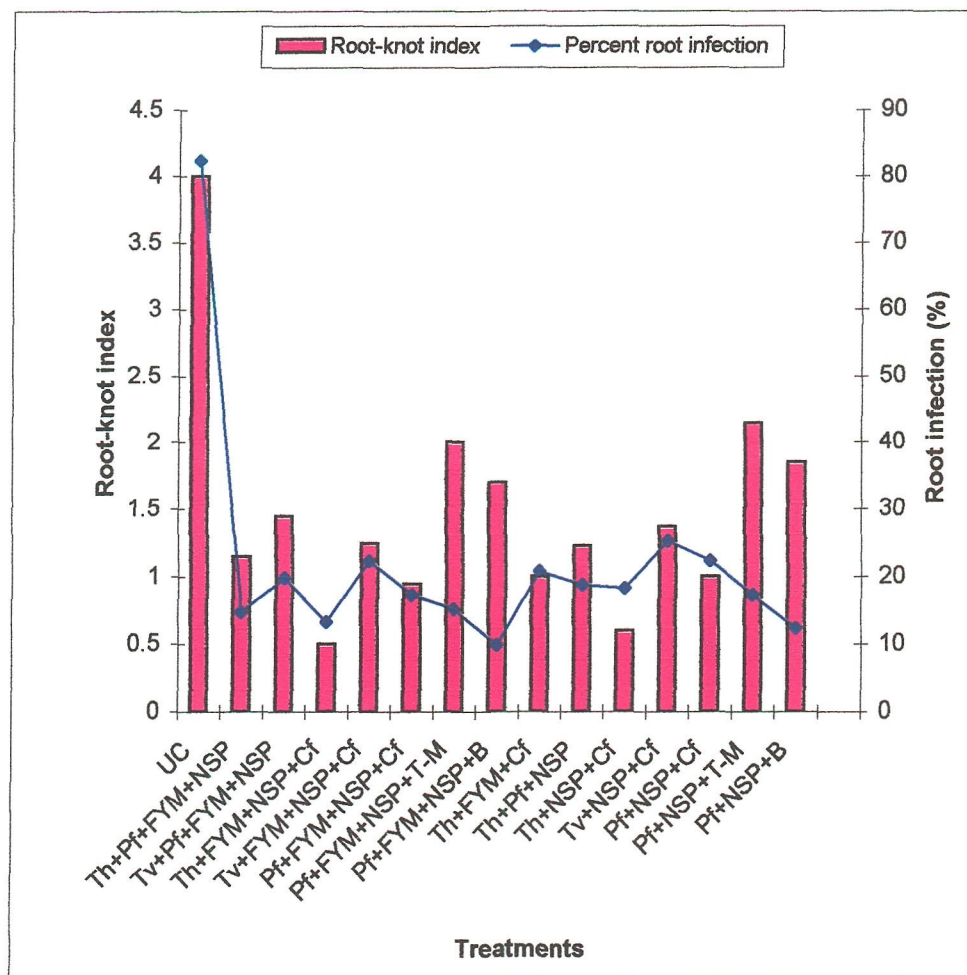


Fig. 19: Comparative efficacy of selected treatments on disease development in tomato cv. K-25 grown in sick field

UC = Untreated control
 Th = *Trichoderma harzianum*
 Tv = *T. virens*
 Pf = *Pseudomonas fluorescens*
 FYM = Farmyard manure

NSP = Neem seed powder
 Cf = Carbofuran
 T-M = Topsin-M
 Bv = Bavistin



Plate 21: Comparative efficacy of selected treatment combinations on the plant growth and yield of tomato cv. K-25 grown in a sick field infested with *M. incognita*, *F. oxysporum* and *R. solani*

(A) A view of the experimental field

(B) A view of best treatment combination with untreated control

1= Untreated control 2= Treated with Th + FYM + NSP + Cf

seed powder + carbofuran (one fourth doses), *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses), *T. harzianum* + neem seed powder + carbofuran (one third doses), *T. virens* + farmyard manure + neem seed powder + carbofuran (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + topsin-M (one fourth doses), *P. fluorescens* + neem seed powder + carbofuran (one third doses), *P. fluorescens* + neem seed powder + bavistin (one third doses), *T. virens* + neem seed powder + carbofuran (one third doses), *T. harzianum* + *P. fluorescens* + neem seed powder (one third doses), *T. virens* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + neem seed powder + topsin-M (one third doses) and *T. harzianum* + farmyard manure + carbofuran (one third doses), respectively (Fig. 18). Similarly, highest reduction in nematode multiplication (0.34) and RKI (0.50) was also found in combined treatment with *T. harzianum* + farmyard manure + neem seed powder + carbofuran (one fourth doses). However, highest reduction in PRI (10.0) was found in combined treatment with *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses) (Fig. 19).

Laboratory, pot, nursery and field studies regarding the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 indicated that this disease complex could effectively be managed to enhance the tomato yield by the application of *T. harzianum* + farmyard manure + neem seed powder + carbofuran (one fourth doses) or *P. fluorescens* + farmyard manure + neem seed powder + carbofuran (one fourth doses) or *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses). Therefore, these components of treatments may safely be recommended to popularize among the farmers for the management of nematode wilt or root rot disease complex for safe and sustainable cultivation on commercial scale to increase the profit.

CHAPTER - 5

DISCUSSION

Under natural conditions, the plants are exposed to various pathogenic microorganisms which can influence each other by occupying the same ecological niche and eventually resulting the diseases of complex etiology. Plant parasitic nematodes are themselves capable of causing severe yield losses, but when soil is infested with other pathogenic organisms in addition to nematodes, the damage on plants becomes manifold (Powell, 1971a, b, 1979; Haseeb, 1983; Webster, 1985; Evans and Haydock, 1993; Back *et al.*, 2002; Luc *et al.*, 2002). The amount of damage caused by nematodes is reported to be influenced by a number of factors such as species of nematode, size of nematode population, host susceptibility, environmental factors and presence of other organisms (Wallace, 1969; Webster, 1972; Norton, 1978; Haseeb, 1983; Back *et al.*, 2002; Luc *et al.*, 2002; Sharma *et al.*, 2005 b).

Having realized the significance of soil borne diseases incited due to complex etiology of nematodes and fungi, the present attempt was made to study the several aspects of interaction of *M. incognita*, *F. oxysporum* and *R. solani* disease complex in tomato and also to develop the management options for their use in an integrated manner under field conditions. The results, thus, obtained are discussed here as under:

1. Survey for the occurrence, distribution and identification of nematode and fungi, causing disease complex in tomato, and for collection of naturally occurring fungal and bacterial antagonists from western districts of Uttar Pradesh

Extensive field surveys carried out in four districts viz., Aligarh, Bulandshahar, Mathura and Agra, revealed the symptoms of disease complexes in tomato fields due to association of root-knot nematodes and wilt and root-rot fungi, particularly *M. incognita* and *F. oxysporum* and *R. solani*. The symptoms of disease complexes noted at different growth stages of crop were characterized with patchy appearance of the field. In most of the locations, the common visual symptoms of affected plants included yellowing of leaves followed by stunting and wilting of plants. In some localities, severely infected plants showed yellow to brown discolouration of leaves with stunted growth and reduction in number and size of fruits, probably due to collar rot and wilting. From most

of the localities, the diseased plants when uprooted visualized with low to severe galling on roots along with the symptoms of wilting and collar or root rot. Young seedlings or plants at early stage of transplanting, exhibited the symptoms of wire stem, collar rot, root rot and wilting along with galling due to root-knot nematodes. However, in some localities, the roots of affected plants were found rotten due to severe infection of either nematode or fungi and both. This symptomatic variation may be accounted for mix inocula of *M. incognita* and *F. oxysporum* and *R. solani*. Such variations in the appearance of symptoms due to association of root-knot nematode and wilt or root rotting fungi, have also been reported by other workers in disease complexes of tomato and other crops (Gallo Llobet *et al.*, 1988; Alabouvette *et al.*, 1998; Haseeb, 2003).

In the present study, the nematode population from diseased plants of tomato was composed of a diverse group of genera, but *Tylenchorhynchus* spp. was dominated in all locations, except in Aligarh, followed by *Meloidogyne* spp., *Hoplolaimus* spp., *Rotylenchulus* sp., *Helicotylenchus* spp. and *Xiphinema* spp. In general, the infected plants from all locations revealed higher root-knot index due to *Meloidogyne* spp., but it was maximum in district Mathura followed by Aligarh, Bulandshahar and Agra. Such plants more frequently yielded the infestation by *M. incognita*, which was more prevalent than *M. javanica*. The identification of fungi revealed the highest percent root infection due to *F. oxysporum* and *R. solani* in district Agra, followed by Aligarh, Bulandshahar and Mathura. The infected plants of tomato often resulted the growth of *F. chlamydosporium*, *F. oxysporum*, *R. solani*, *F. solani*, *Pythium aphanidermatum* and *P. ultimum*, but *F. oxysporum* and *R. solani* were frequently yielded from the infected plants. The associations of these fungi have also been reported from wilt and root rot infected plants of tomato from different other growing areas (Kapoor, 1988; Dolar, 1996; Arora and Gupta, 2002; Haseeb, 2003; Mathur *et al.*, 2004). These findings clearly indicate that population of root-knot nematode and their incidence vary with localities, which might be due to variation in susceptibility of tomato cultivars, soil types and other cultural practices adopted by the farmers (Prot and Van Gundy, 1981; Swanson and Van Gundy, 1984; Southey, 1986). In addition, several other factors may also influence the amount of damage due to disease complexes, which are reported to be dependent on species and population density of nematodes, presence of other pathogenic organisms,

host plant susceptibility and environmental factors (Powell, 1971 a, b; Norton, 1978; Lamberti and Taylor, 1979; Veech and Dickson, 1987).

2. Pathogenicity test of *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani* alone on tomato cv. K-25 under pot conditions

All three causal pathogens of disease complex i.e., *M. incognita*, *F. oxysporum* and *R. solani* showed their pathogenic potential and reduced plant growth and fruit yield of tomato cv. K-25. In general, there was a positive relationship between initial inoculum level (Pi) of *M. incognita* and reduction in all the test parameters and a negative relationship between initial inoculum densities and rate of nematode multiplication (Rf). Similar relationship between Pi of *M. incognita* and growth parameters has also been reported by several other workers (Barker *et al.*, 1976; Di Vito *et al.*, 1983, 1985; Butool and Haseeb, 1998; Haseeb *et al.*, 2005 b; Perveen *et al.*, 2006). The reduction in reproduction factor with corresponding increase in Pi is attributed to the destruction of root system, competition for nutrition among the developing nematodes within a root system, inability of larvae of subsequent generations to locate the infection sites (Triantaphyllou and Hirschmann, 1960; Wallace, 1971; Apple and Lewis, 1984; Daiber, 1989, 1990; Ehwaeti *et al.*, 1998; Haseeb, 2003; Haseeb *et al.*, 2005 b).

As similar to the observations recorded in pathogenicity test of *M. incognita*, the increasing inoculum levels of *F. oxysporum*, except Pi of 2.5×10^6 cfu/5 kg soil, also exhibited a gradual reduction in plant length, fresh and dry weight of tomato cv. K-25. These results are in general agreement with the work of Yousef *et al.* (1992). Ramezani *et al.* (1999), Haseeb (2003) and Sharma *et al.* (2005 a), who had the similar views on the relationship between initial inoculum level of *F. oxysporum* and severity of disease. The pathogenicity test of *R. solani* also revealed an increase in root infection and decrease in plant growth and fruit yield with the increasing inoculum levels of *R. solani*. The similar reports are also available on the direct relationship between inoculum concentration and disease severity of *R. solani* on various host crops (Warren, 1975; Haseeb, 1983; Otrysko and Banville, 1992; Keinath, 1995; Tyagi *et al.*, 2005).

It is clear from the observations that the lowest initial inoculum levels of *F. oxysporum* (2.5×10^6 cfu/5 kg soil) and *R. solani* (2.5 g mycelium/5 kg soil) may result

significant damage to tomato cv. K-25. These inoculum levels could safely be used as an additional criterion to assess the expected losses due to wilt and root rot and to develop the management options against these pathogens under field conditions.

3. Interactive effect of *M. incognita*, *F. oxysporum* and *R. solani*, alone and in combination on disease development, plant growth and fruit yield of tomato cv. K-25 under pot conditions

Evidences are documented that when root-knot nematode and fungus interact on a host, damage varies with the sequence of inoculations. In the present study, the effect of sequential, simultaneous and single inoculation with *M. incognita*, *F. oxysporum* and *R. solani* was tested on tomato cv. K-25. In general, *M. incognita* was more pathogenic than *R. solani* or *F. oxysporum* alone in reducing plant growth and fruit yield of tomato cv.K-25. However, maximum reduction of growth and yield parameters was observed in plants simultaneously inoculated with *M. incognita*, *F. oxysporum* and *R. solani*, followed by *M. incognita* prior to *F. oxysporum* and *R. solani*, *F. oxysporum* and *R. solani* prior to *M. incognita*, *M. incognita* and *R. solani* simultaneously, *M. incognita* prior to *R. solani*, *M. incognita* and *F. oxysporum* simultaneously, *M. incognita* prior to *F. oxysporum*, *F. oxysporum* and *R. solani* simultaneously, *R. solani* prior to *M. incognita*, and *F. oxysporum* prior to *M. incognita*, respectively. However, root-knot index was highest in plants inoculated with nematode alone, yet maximum root infection resulted due to simultaneous inoculations of nematode and fungi.

These findings are in strong agreement with those of others, who noted that when root-knot nematode and fungus infect on a host, the corresponding damage vary with the sequence of inoculations (Singh *et al.*, 1991; Shah *et al.*, 1993; Back *et al.*, 2002).Conversely, fungal root infection has also been found to be increased in presence of nematodes in various crops including tomato, because the roots are predisposed by *M. incognita* for further damage by *F. oxysporum* and *R. solani* (France and Abawi, 1994). Perveen *et al.* (1999 a, b) also expressed the similar views on disease complex of *M. incognita* and *R. solani*, using okra and tomato as host crops. Whereas, Golden and Van Gundy (1975) hypothesized that leakage of nutrients from the root could be an another factor responsible for attracting the fungus towards the galled roots induced by nematode. In addition, the root-knot nematodes provide the infection courts through which the fungi

facilitate their entry as reported by the other workers (Haseeb, 1983; Singh *et al.*, 1991; Luc *et al.*, 2002; Haseeb, 2003, 2004; Sharma *et al.*, 2005 b).

4. In-vitro, evaluation of biocontrol agents, organic amendments and pesticides for their efficacy against *F. oxysporum* and *R. solani* alone

In dual culture, screening of native biocontrol agents revealed highest antagonistic potential of isolates TH-H-3, TV-K-3 and PS-4 of *T. harzianum*, *T. virens* and *P. fluorescence*, respectively against *F. oxysporum* and *R. solani* alone. These isolates inhibited the maximum mycelial growth of both tested pathogens and proved to be more promising in their efficacy. The reports available on the antagonistic effect of *Trichoderma* spp. against these pathogens indicate various mechanisms of action, including mycoparasitism, competition and antibiosis (Dennis and Webster, 1971; Haseeb, 2003; Kucuk and Kivanc, 2003; John *et al.*, 2006; Haseeb *et al.*, 2007 b). On the other hand, the efficacy of *P. fluorescens* against target pathogens is claimed also due to production of certain antibiotic compounds like pyoluteorin (Howell and Stepanovic, 1979) and pyrrolnitrin, pyocyanine, 2,4- diacetylphloroglucinal (Pierson and Thomashow, 1992). However, Kloepper *et al.* (1980) advocated for the production of siderophores by *P. fluorescens* which limit the availability of iron, thus, resulting reduction in growth of the pathogens. Several other reports on the antagonistic effect of *Trichoderma* spp and *P. fluorescens* against test pathogens are also available (Kim and Roh, 1987; Fravel, 1988; Selvarajan and Jeyarajan, 1996; Rajappan and Ramraj, 1999; Haseeb, 2003; Haseeb *et al.*, 2006 a, 2007 b; John *et al.*, 2006).

In dual culture, pesticides (carbofuran, bavistin and topsin-M) and organic amendments (neem seed powder, farmyard manure) also exhibited their promising effect in reducing the mycelial growth of both the tested pathogens. These results are also in support of earlier reports of Nene and Thapliyal, (2000) and Thind and Chahal (2002), who opined that these fungicides interfere with nuclear division and biosynthesis of new cell material of fungi that requires for growth and maintenance. Evidences are also documented with regard to the efficacy of farm yard manure and neem products against many soil borne pathogens (Smith and Ashworth, 1965; Shivpuri *et al.*, 1997; Sindhan *et al.*, 1999; Haseeb, 2003; Haseeb *et al.*, 2005 a; Maurya *et al.*, 2008).

5. Comparative efficacy of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* alone and in combinations, on tomato cv. K-25 under pot conditions

After evaluation of various treatments, in-vitro, the comparative efficacy of these treatments viz., biocontrol agents, pesticides and organic amendments were then evaluated in pot conditions, using *M. incognita*, *F. oxysporum*, *R. solani* alone and in different combination of inoculations, i.e. *M. incognita* alone, *F. oxysporum* alone, *R. solani* alone, *F. oxysporum* and *R. solani* simultaneously, *M. incognita* and *F. oxysporum* simultaneously, *M. incognita* and *R. solani* simultaneously and *M. incognita*, *F. oxysporum* and *R. solani* simultaneously.

5.1 *M. incognita* alone

All treatments resulted an increase in plant growth and fruit yield of tomato cv. K-25 in pots inoculated with *M. incognita* alone, however, carbofuran was found most effective followed by neem seed powder and *T. harzianum* (TH-H-3), in increasing the growth and yield and reducing the multiplication of *M. incognita*. The efficacy of these treatments, though, was noted to be at par when compared from each other. The efficacy of these treatments corroborates with the reports of other workers (Singh and Sitaramaiah, 1973; Haseeb, 1983; Alam, 1991; McSorley *et al.*, 1997; Siddiqui *et al.*, 1999; Sharon *et al.*, 2001; Haseeb *et al.*, 2006 b). Earlier Wright (1981) had an opinion that carbofuran, a systemic and granular nematicide with non-fumigant action does not cause direct mortality, but affects the nematodes through contact in soil. Besides, it also gets absorbed in plant system and thus, interferes with feeding of nematodes. It also inhibit acetyl-cholinesterase at the nerve synapse, causing malfunctioning of the muscular and other organic systems of the nematode. This disruption of these systems, therefore, influences the nematode movement, behaviour and ultimately alters the infection process of the parasitism, either by delaying or reducing the penetration (Hertwig and Sikora, 1991).

Neem products viz., cake, leaf extract, seed powder contain several active principles and hamper the infection process of pathogenic fungi, because after decomposition several chemical compounds are released which may have fungicidal and nematicidal properties that operate to lead the reduction in population of pathogenic

organisms (Narayanan and Ayer, 1967; Barak and Chakraborty, 1969; Schippers and Palm, 1973). These also possess nemato-toxic substances like ammonia, phenols and aldehydes, affecting the pathogens (Miller and Edington, 1962; Alam, 1991). The possible mechanism of action of *T. harzianum* against root-knot nematode is not yet well studied, however, this bioinoculant is known to produce hydrolytic enzymes including, chitinases, β -1, 3-glucanases, proteases and other volatile and non-volatile compounds (Lumsden and Locke, 1989). In addition, Bird and Self (1995) demonstrated that eggshell of nematodes contains chitin, which they also detected from gelatinous matrix of egg masses of the root-knot nematode. In general, the reduction in root-knot nematode population is attributed to the parasitization of egg, eggmasses and larvae by the antagonistic fungi, *T. harzianum* is also accounted for the production of hydrolytic enzymes such as, chitinases, endochitinases, proteases, etc., but the differences in the parasitization levels are found to be dependent on varying levels of hydrolytic enzymes produced by the individual species and strains. These antagonistic fungi not only inhibit egg hatching but also cause mortality of *M. incognita* juveniles (Goswami and Rao, 1997; Nagesh *et al.*, 2001; Sharon *et al.*, 2001; Haseeb *et al.*, 2005 c, d).

5.2. *F. oxysporum* alone

The various treatments comprised of biocontrol agents, organic amendments and pesticides were found to reduce percent root infection, thereby enhancing the plant growth and fruit yield of tomato cv. K-25 in pots inoculated with *F. oxysporum*. Of all treatments, *T. harzianum* (TH-H-3) proved to be most effective followed by bavistin, *P. fluorescens* (PS-4), topsin-M and *T. virens* (TV-K-3). However, all these treatments did not show significant differences in their efficacy against *F. oxysporum*, when compared from one another. *Trichoderma* spp., when applied as seed and soil treatment provide long term protection against soil borne pathogens and enhance the plant growth (Papavizas, 1985; Ahmad and Baker, 1987 a, b; Windham *et al.*, 1989; Singh *et al.*, 2004 a). Earlier, Elad (1995) also advocated for the various mechanisms responsible for the efficacy of *Trichoderma* spp against phytopathogenic fungi. The disease controlling efficacy of *Trichoderma* spp. is also due to their ability to grow rapidly and to colonize the infection courts, thus, competing with the pathogens in soil (Hjeljord and Tronsmo,

1998). The present findings are similar to the results obtained by various workers with the use of these treatments against *F. oxysporum* in many other crops, including tomato (Sivan and Chet, 1989; Rajappan and Ramraj, 1999; Padmodaya and Reddy, 1999; Kumar and Dubey, 2001; Haseeb *et al.*, 2006 a; Devi *et al.*, 2008; Maurya *et al.*, 2008). The efficacy of bavistin observed in the present study against *F. oxysporum*, has also been reported by several other workers (Etabarian *et al.*, 1992; Javed *et al.*, 1997; Bharath *et al.*, 2005; Sarkar and Saxena, 2007; Devi *et al.*, 2008), who interpreted that when this fungicide comes in contact with the plant it gets converted at the plant surface to methyl benzimidazole carbamate, a compound responsible for interfering with nuclear division and biosynthesis of new cell of the fungi.

However, the antagonistic potential of *P. fluorescens* (PS-4) against *F. oxysporum* could be attributed to the production of antibiotics (Fravel, 1988), siderophore production (Schippers *et al.*, 1987; Bakker *et al.*, 1993) and due to induced systemic resistance (Kloepper *et al.*, 1980; Van Peer *et al.*, 1991). Besides these, suppression of root pathogens is also due to competition for food and ability to colonize the roots (Park, 1990). Moreover, *P. fluorescens* produces plant growth promoting substances, thereby enhancing plant growth and yield (Burr *et al.*, 1978; Kaiser *et al.*, 1989; Sarvanan *et al.*, 2004). Several strains of *P. fluorescens* have been reported to be effective against various species of Fusarium, including *F. oxysporum* f. sp. *ciceri* in chickpea (Vidhyasekaran and Muthamilan, 1995), *F. oxysporum* f. sp. *radicis lycopersici* in tomato (M'Piga *et al.*, 1997), *F. moniliforme* in cauliflower (Rajappan and Ramraj, 1999), *F. solani* in tomato (Haseeb, 2003) and *F. oxysporum* in chilli (Haseeb *et al.*, 2006 a) and brinjal (Haseeb *et al.*, 2007 a).

5.3. *R. solani* alone

All treatments showed their effectiveness in improving the growth parameters of tomato cv. K-25 in pots inoculated with *R. solani* alone, but *T. harzianum* (TH-H-3) resulted maximum increase in plant growth and fruit yield followed by topsin-M, *P. fluorescens* (PS-4), bavistin, *T. virens* and neem seed powder. Fortunately, fungal inoculants (TH-H-3 and TV-K-3) and bacterial inoculant (PS-4) exhibited insignificant differences in their antagonistic potential against *R. solani*. The efficacy of these

treatments against *R. solani* has also been reported by other workers (Elad *et al.*, 1980 a; Chet and Baker, 1981; Howell and Stipenovic, 1995; Howell, 1991; Howell *et al.*, 1993; Papavizas and Collins, 1990; Park *et al.*, 1992; Di Pietro *et al.*, 1993). The mode of action of *T. harzianum* has already been discussed in preceding paragraphs. Besides, these mycoparasitic fungi are able to attack sclerotia or mycelium soil borne of the pathogens and reduce inoculum in the soil (Rahman *et al.*, 2001; Wagner and Kopacki, 2002; Bandyopadhyay *et al.*, 2003; Gandhi and Kumar, 2006). On the other hand, the fungitoxic activity of topsin-M against *R. solani* has been claimed due to its conversion into methyl-2-benzimidazole carbamate (MBC), it may be in response to its transformation to ethyle-2-benzimidazole carbamate (EBC). However, the MBC is the actual fungitoxic moiety of topsin-M. According to Selling *et al.* (1970) and Buchenauer (1975), this fungicide also acts by interfering in DNA synthesis/nuclear or cell division. Whereas, Kataria and Grover (1976) opined that topsin-M causes inhibition of respiration and synthesis of DNA. Also, Ho *et al.* (1992) holds the similar view with respect to the fungitoxicity of this compound, as noted in the present study.

Several compelling reasons for the efficacy of Fluorescent Pseudomonads against soil borne plant pathogens has already been discussed in the preceding paragraphs, in accordance with the available reports (Kloepper *et al.*, 1980; Weller, 1988; Mazzola and Cook, 1991; O'Sullivan and O'Gara, 1992; Chen *et al.*, 1995).

5.4. *F. oxysporum* + *R. solani*

All treatments were found effective and showed an increase in plant growth and fruit yield of tomato in pots inoculated simultaneously with *F. oxysporum* and *R. solani*. However, the application of *T. harzianum* (TH-H-3) gave maximum increase in plant growth and fruit yield followed by bavistin, *P. fluorescens* (PS-4), *T. virens* (TV-K-3) and topsin-M, yet maximum reduction in root infection was resulted with bavistin followed by topsin-M, *T. harzianum* (TH-H-3), *P. fluorescens* (PS-4). The differences among the efficacy of biocontrol agents on one hand and among the bavistin and topsin-M on the other hand, was found to be insignificant against mixed inocula of *F. oxysporum* and *R. solani*. The effectiveness of these treatments, noted under study, has also been reported by several other workers (Kloepper *et al.*, 1980; Schippers *et al.*,

1987; Fravel, 1988; Chakraborti and Sen, 1991; Elad, 1995; Hjeljord and Tronsmo, 1998; Padmodaya and Reddy, 1999; Sabet *et al.*, 2000; Thind and Chahal, 2002; Haseeb, 2003; Bharath *et al.*, 2005; Haseeb *et al.*, 2005 a, 2006 a ; Haseeb and Kumar, 2007 b; Haseeb and Kumar, 2007 b; Maurya *et al.*, 2008).

5.5. *M. incognita* + *F. oxysporum*

In simultaneous inoculation with a root knot nematode *M. incognita* and wilt causing fungus *F. oxysporum* on tomato cv. K-25 in pots, all treatments of biocontrol agents, organic amendments and pesticides considerably increased plant growth, fruit yield and reduced the root-knot development, *M. incognita* reproduction and root infection by *F. oxysporum*. Here too, *T. harzianum* (TH-H-3) gave maximum increase in growth and yield parameters of tomato plants followed by carbofuran, *P. fluorescens* (PS-4), bavistin and *T. virens* (TV-K-3), but all these treatments did not differ statistically in their efficacy when compared from one and another. The present findings are, however, in conformity with the observations recorded on the antagonistic activity of *T. harzianum* (TH-H-3) in previous experiments in this study, wherein pots inoculated with *M. incognita* and *F. oxysporum* alone, and also with earlier reports of several other workers who noted the similar effect of this bioinoculant against these pathogens in various other crops, including tomato (Jones and Overmann, 1976; Parveen *et al.*, 1993; Sinha and Mukhopadhyay, 1993; Singh and Goswami, 2001; Haseeb, 2003; Haseeb *et al.*, 2004, 2005 c, d; Haseeb and Kumar, 2008 a, c).

Contrary to this, carbofuran was found most effective in reducing nematode population, root-knot index followed by *T. harzianum* (TH-H-3) and *P. fluorescens* (PS-4), whereas, maximum reduction in root infection due to *F. oxysporum* was noted with treatment of bavistin followed by *T. harzianum* (TH-H-3) and topsin-M. With regard to the efficacy of carbofuran, Jones and Overman (1976) opined that carbofuran is not although a fungicide, yet its negative influence should be seen in the light of nematode-fungus interaction. They also noticed an improvement with this pesticide in the yield of tomato affected by nematodes, namely *M. acrita*, *Belanolaimus longicaudatus*, *Trichodorus christiei* and wilt fungi like *F. oxysporum* f. sp. *lycopersici* race 2 and *Verticillium albo-atrum*. The reports are also available on the management of

Meloidogyne-Fusarium disease complex on different other crops (Singh and Goswami, 2001; Haseeb, 2003; Haseeb *et al.*, 2004, 2005 c, d, 2006 b; Haseeb and Kumar, 2005, 2008 a, c).

Kluepfel *et al.* (1993), while assessing the efficacy of *P. fluorescens*, found its effectiveness against disease complex probably due to their antagonistic potential against nematodes. Whereas, Backer *et al.* (1988) found aggressive root colonization by *P. fluorescens* and also noted the production of nematicidal compounds in the suppression of juveniles of *M. incognita*. Thomashow *et al.* (1990) advocated that antibiotic production is an important factor in the disease suppressing ability of *P. fluorescens*. In addition, plant growth promoting hormones like gibberellins, cytokinins and indole-3 acetic acid, produced by this bacterium have also been claimed to contribute for the mechanisms of plant growth promotion and yield improvement (Siddiqui *et al.*, 1998; Khan and Akram, 2000; Haseeb, 2003; Haseeb *et al.*, 2004, 2005 c, d, 2006 b; Haseeb and Kumar, 2005, 2008 a, c).

5.6. *M. incognita* + *R. solani*

In simultaneous inoculations with *M. incognita* and *R. solani*, all treatments revealed their efficacy in increasing the plant growth and fruit yield thereby reducing root-knot development and root infection of tomato plants in pots. All biocontrol agents viz., *T. harzianum* (TH-H-3), *T. virens* (TH-H-3) and *P. fluorescens* (PS-4) and one pesticide i.e., carbofuran resulted maximum increase in growth and yield parameters of tomato, but these treatments show insignificant differences in their efficacy when compared from one and another. However, maximum reduction in the reproduction of *M. incognita* and root-knot development was achieved by carbofuran followed by *T. harzianum* (TH-H-3) and neem seed powder. It is, therefore, clear that among all treatments, *T. harzianum* and carbofuran gave most satisfactory results. The different mechanisms of action of *Trichoderma spp* operated against different soil borne pathogens has already been reported by several workers and discussed earlier in previous experiments (Chet, 1987; Elad, 1995 Mehta *et al.*, 1995; Chand and Tripathi, 2002; Chaitali *et al.*, 2003). Loganathan *et al.*, (2001) also noted the promising effect of *P. fluorescens*, concluding that chitinases and β -1, 3-glucanases, an enzyme responsible to

degrade the chitin and glucon, major components of cell wall of the higher fungi as well as of the exoskeleton and peritrophic membrane of insects and nematodes, respectively. Siddiqui *et al.* (2001) also opined for the efficacy of *P. aeruginosa* against *M. javanica* and *R. solani* disease complex in tomato. During the recent past, Haseeb and Kumar (2008 b) also successfully managed disease complex of *M. incognita* and *R. solani* using *P. fluorescens* in brinjal. Besides nematicidal properties of carbofuran, Abu-El-Amayam *et al.* (1985) observed its fungicidal effect against disease complex of *M. incognita* and *R. solani*. The efficacy of other treatments viz., topsin-M, bavistin, neemseed powder and farm yard manure recorded in the present study have close testimony with the results of previous workers (Sayre *et al.*, 1964; Patrick and Toussoun, 1965; Khan *et al.*, 1973, 1974; Cook, 1977; Sitaramaiah, 1990; Walia *et al.*, 1994; Haque *et al.*, 1995; Arya and Saxena, 1998; Siddiqui *et al.*, 2001; Chand and Tripathi, 2002; Haseeb and Kumar, 2008 b; Haseeb and Kumar, 2008 b).

5.7. *M. incognita* + *F. oxysporum* + *R. solani*

Besides the efficacy of various treatments in other combinations of inoculations, all treatment components here too, in pots simultaneously inoculated with the mixed inocula of disease complex i.e., *M. incognita*, *F. oxysporum* and *R. solani*, resulted in improved plant growth and fruit yield of tomato cv. K-25. Among all treatments, *T. harzianum* and *P. fluorescens* were most promising, though, other effective treatments were carbofuran, *P. fluorescens*, *T. virens* and bavistin, which did not differ statistically from each other. However, carbofuran followed by neem seed powder and *P. fluorescens* gave maximum reduction in root-knot development, reproduction of *M. incognita* and root infection by *F. oxysporum* and *R. solani*. On the other hand, bavistin treated plants showed maximum reduction in root infection due to both the tested pathogens, whereas, the efficacy of other fungicides i.e., topsin-M was found to be statistically at par with that of *T. harzianum*. The efficacy of biocontrol agent is also due to competition for space and nutrients which play an important role in reducing the RKI and root infection by nematode and fungi (Chet, 1987). Several workers also advocated for the possible reasons for reducing root-knot infection due to *Trichoderma* spp. but they also found parasitization of root-knot eggs by lytic enzymes chitinases, production of antibiotics,

namely trichodermin, trichoviridin, dermadin etc., which enhance its biocontrol efficacy against nematodes as well as fungi (Chet and Baker, 1981; Howell, 2003). The biocontrol potential of *Trichoderma* spp. against multipathogenic disease complex has also been noticed by Mousa and Mousa (1994) and Siddiqui *et al.* (1999). The ameliorating effects of biocontrol agents, namely *Trichoderma* spp., *P. fluorescens*, and the effectiveness of other treatments recorded in the present study have also been reported by various other workers against disease complex in different crops (Weller, 1988; Mousa and Mousa, 1994; Haque *et al.*, 1995; Mehta *et al.*, 1995; Siddiqui *et al.*, 1999; Siddiqui, 2000).

6. Compatibility test, *in-vitro*, among fungal and bacterial biocontrol agents and thereof with pesticides and organic amendments

In this study, promising isolates of *T. harzianum* (TH-H-3) and *T. virens* (TV-K-3) were found compatible with *P. fluorescens* (PS-4), showing 100 and 77.8% compatibility, respectively. On the other hand, *Trichoderma* spp isolates showed their compatibility with all other component of treatments, except fungicides (topsint-M and bavistin), conversely it was noteworthy that *P. fluorescens* (PS-4) showed their compatibility with fungicides, pesticides and other organic amendments. These findings regarding the compatibility of *P. fluorescens* are also in strong agreement with Jeong *et al.* (1993) and Haseeb (2003), who observed the compatibility of *P. putida* with *G. virens* G 872 and of *P. fluorescens* with *T. harzianum* and *T. virens*, respectively. Siddiqui and Shaukat (2004), while experimenting on compatibility of strains of fungal and bacterial bioinoculants, also observed the success between *T. harzianum* strain Th 6 and *P. fluorescens* strain CHA0. The similar observations recorded on the compatible reaction among the *Trichoderma* spp. in the present study have also been reported by Alvarez *et al.* (1995), Khan (2000), Kumar and Dubey (2001), Pant and Mukhopadhyay (2001), Haseeb (2003), Mahapatra and Mohanty (2003), Chaitali *et al.* (2003), Haseeb (2003) and Zaidi and Singh (2004). These findings are also in confirmatory with other reports, wherein the incorporation of organic matter, like farmyard manure or neem products, *viz.*, seed powder, cake or leaf extract, has been resulted in increased population of *Trichoderma* spp. in the soil. The availability of organic matter in soil provides an effective substrate for growth, multiplication and stimulation of sporulation of

antagonistic organisms thereby enhancing their activity (Sheela *et al.*, 1995; Haseeb, 2003).

7. Efficacy of biocontrol agents, organic amendments and pesticides alone and in combination, against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in nursery under sick plot conditions

All treatments were found to be compatible, *in-vitro*, when evaluated alone or in various combinations against disease complex of tomato under sick plot conditions in nursery and showed their effectiveness in improving the percent emergence, fresh weight of seedling and reducing the disease incidence. Among the treatments used alone, bavistin was found most effective resulting highest reduction in root infection and improving the percent emergence and fresh weight of seedlings, however maximum reduction in root-knot index was recorded with carbofuran, followed by neem seed powder, *T. harzianum* and bavistin. In combinations of treatment, when all three biocontrol agents i.e. *T. harzianum* (TH-H-3), *T. virens* (TV-K-3) and *P. fluorescens* (PS-4) were used individually to get the combinations with organic amendments and pesticides, also showed their efficacy, being the best with the application of two additives i.e., *P. fluorescens* (PS-4) + bavistin. Though, it was interesting to note that both fungal bioinoculants resulted in maximum percent emergence and fresh weight of seedlings and also gave highest reduction in root knot index, yet maximum reduction in percent root infection was possible only when *P. fluorescens* (PS-4) was combined with either of both isolates of *Trichoderma* spp. Contrary to this, when all three bioinoculants were combined with each other, *T. harzianum* (TH-H-3) proved to be more effective than *T. virens* (TV-K-3) and *P. fluorescens* (PS-4). These findings are in agreement to those reported by others (Steinmetz and Schonbeck, 1994; Lewis *et al.*, 1998; Hoitink and Boehm, 1999; Howell *et al.*, 2000; Harman *et al.*, 2004). They found the differences in the potential of fungal and bacterial biocontrol agents with the varied spectrum, type and amount of antibiotic production.

In case of integration of three additives, the treatment with *T. harzianum* (TH-H-3) + neem seed powder + carbofuran proved best in increasing the number of emerging seedlings, their fresh weight and in reducing the disease incidence, whereas, in four

additives it was possible after adding farm yard manure i.e., *T. harzianum* (TH-H-3) + neem seed powder + carbofuran + farm yard manure. The promising results in the study were, however, obtained when isolates of either *T. harzianum* or *T. virens* or *P. fluorescens* was applied in integration with farmyard manure, neem seed powder, carbofuran and bavistin or topsin-M against *Meloidogyne-Fusarium-Rhizoctonia* disease complex in tomato.

The present study, therefore, suggests that the efficacy of biocontrol agents is further increased when they are applied simultaneously in combined treatments with organic amendments (neem seed powder and farmyard manure) and pesticides (carbofuran, bavistin and topsin-M). Earlier, Stephan *et al.* (1996) also noticed an increase in the efficacy of *Trichoderma* spp. in increasing plant growth and yield of tomato against *M. javanica* + *F. oxysporum* f. sp. *lycopersici* disease complex, when applied in combination either with *P. lilacinus* or oxamil or fenamiphos or benomyl. Subsequently, Siddiqui *et al.* (1999) observed most effective integrated control for *M. javanica* + *F. solani* + *R. solani* disease complex on tomato with combined application of *Trichoderma* spp. and *P. aeruginosa*. Similarly, the application of *T. viride* with neem cake was found most effective against *M. incognita* + *M. phaseolina* disease complex in okra (Chaitali *et al.*, 2003). Further, Haseeb *et al.* (2003 b) and Haseeb *et al.* (2005 c) noted an increase in the efficacy of application of *T. harzianum* with carbofuran or carbofuran with topsin-M against root-knot-wilt disease complex on chilli in nursery and application of *T. harzianum* (full dose) with carbofuran (half dose) against *M. incognita* + *F. oxysporum* disease complex on brinjal.

8. Evaluation of various treatments of biocontrol agents, organic amendments and pesticides against *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25 in sick field conditions

The effect of selected treatment combinations was also studied for the management of disease complex of *M. incognita*, *F. oxysporum* and *R. solani* in tomato cv. K-25 under field conditions. The observations indicated that, in general, all treatments improved plant growth, fruit yield and suppressed infection due to nematode and fungi, when compared to untreated control. The maximum increase in plant weight and fruit yield, and decrease in RKI and root infection was observed in plots treated with the

combination of *T. harzianum* + farmyard manure + neem seed powder + carbofuran followed by the *P. fluorescens* + farmyard manure + neem seed powder + carbofuran, *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder, *P. fluorescens* + farmyard manure + neem seed powder + bavistin, *T. harzianum* + neem seed powder + carbofuran, *T. virens* + farmyard manure + neem seed powder + carbofuran, *P. fluorescens* + farmyard manure + neem seed powder + topsin-M, *P. fluorescens* + neem seed powder + carbofuran, *P. fluorescens* + neem seed powder + bavistin, *T. virens* + neem seed powder + carbofuran, *T. harzianum* + *P. fluorescens* + neem seed powder, *T. virens* + *P. fluorescens* + farmyard manure + neem seed powder, *P. fluorescens* + neem seed powder + topsin-M and *T. harzianum* + farmyard manure + carbofuran.

There are enormous evidences regarding the integrated management of disease complexes of soil borne pathogens with the use of biocontrol agents, organic amendments and pesticides, which not only suppress the pathogens but also improve health and growth of the plants, thereby resulting an increase in yield of plants. The results of comparative efficacy of different treatment combinations recorded against *M. incognita*, *F. oxysporum* and *R. solani* under field conditions has visualized the close similarity with that those noted in the nursery. Therefore, these findings further confirm the efficacy of all treatment combinations of biocontrol agents with organic amendments and pesticide against this disease complex in tomato. Several workers have also reported the successful management of nematode and fungus disease complexes, using the similar component of treatments alone or in combinations (Stephan *et al.*, 1996; Siddiqui *et al.*, 1999; Chaitali *et al.*, 2003; Haseeb, 2003; Haseeb *et al.*, 2003 b, 2005 c, d, Haseeb and Kumar, 2008 a, c).

On the basis of overall results obtained in this study, the application of *T. harzianum* *P. fluorescens* (25 kg/ha) + farmyard manure (750 kg/ha) + neem seed powder (25 kg/ha) + carbofuran (16.5 kg /ha) could be recommended for the management the disease complex of *M. incognita*, *F. oxysporum* and *R. solani* in tomato crop under field conditions. Besides, in the present scenario of ill effect of pesticides, we may apply *T. harzianum* (25 kg/ha) and *P. fluorescens* (25 kg/ha) with organic amendments like farmyard manure (750 kg/ha) + neem seed powder (25 kg/ha). The unison of all these treatment combinations, comprising of biocontrol agents, organic amendments and

pesticide would be an economical and sustainable approach of disease complex of nematode and fungi in tomato crop under field conditions.

It could further be inferred from the above results that among the comparative efficacy of different treatments i.e. biocontrol agents, organic amendments and pesticides, the application of carbofuran, *T. harzianum* isolate TH-H-3 and *T. harzianum* isolate TH-H-3 was most efficacious in improving the growth parameters and reducing root infection in tomato cv. K-25 plants inoculated with *M. incognita*/ *F. oxysporum*/ *R. solani* alone, respectively.

This study, therefore, suggests that the application of *T. harzianum* in combination with neem seed powder, topsin-M and Bavistin in integrated manner could be of greater significance for effective management of root-knot nematode - wilt and root-rot disease complex of tomato under field conditions.

CHAPTER - 6

SUMMARY

Tomato (*Lycopersicon esculentum*) is one of the most important crops and outranks all other vegetables except the potato crop in popularity and value in the world. This crop has been suffered with plant parasitic nematodes, particularly root-knot nematodes (*Meloidogyne* species) and soil borne fungi, especially *Fusarium* and *Rhizoctonia* spp. resulting in heavy yield loss. Keeping in view the importance and ever increasing demand of tomato and the damaging potential of root-knot nematodes alone and in combination with several soil borne fungi, this study was carried out to generate information pertaining to the survey of tomato growing areas in western Uttar Pradesh for the distribution and severity of diseases caused by plant-parasitic nematodes and soil borne fungi, particularly regarding the singular and combined effect of *M. incognita*, *F. oxysporum* and *R. solani* on the growth and fruit yield of tomato cv. K-25, a favorite variety of farmers and consumers in this region. The effects were also made to develop an eco-friendly, economically and sustainable package of Integrated Disease Management practices to offset the losses caused by the disease complexes.

In order to assess the distribution of the plant parasitic nematode-fungal disease complexes of tomato, an extensive survey was conducted during the month of April, 2004 and March, 2005 in farmers' fields growing tomato in Agra, Aligarh, Bulandshahar and Mathura districts of Uttar Pradesh. Symptomalogical studies indicated the patchy appearance of the disease which included yellowing of leaves, chlorosis and wilting of the plants, which was commonly noticed in all the localities surveyed. Collar rot with wire stem was also observed with wilting at nursery and early stages of crop growth in most of the localities. However, occasionally low to severe galling was also observed in roots of affected plants. Field samples studied in the laboratory more often revealed the presence of *F. oxysporum* and *R. solani*. However, *F. solani*, *F. chlamydosporium*, *M. phaseolina*, *P. ultimum* and *P. aphanidermatum* were also isolated from such infected plants. The highest percent root infection by fungi was found in Agra followed by Aligarh, Bulandshahar and Mathura district, respectively.

Besides soil borne fungi, several important plant parasitic nematodes such as larvae of *Meloidogyne*, *Tylenchorhynchus*, *Hoplolaimus*, *Helicotylenchus*, *Rotylenchulus* and *Xiphinema* spp. were also isolated from the rhizospheric soil of diseased plants of tomato grown in these localities. However, the population of *Pratylenchus*, *Tylenchus*, *Heterodera*, *Longidorus* and *Criconimoides* spp. were also occasionally yielded in isolations. Studies regarding the identification of species of root-knot nematodes from the galled roots indicated the presence of *M. incognita* singly in most of the localities surveyed. However, mixed infestation of *M. incognita* and *M. javanica* was also encountered from the same root system of infected plants collected during the survey. The highest population of larvae of *Meloidogyne* spp. in soil and also root-knot index was found in Mathura followed by Aligarh, Bulandshahar and Agra district, respectively.

During the survey attempts were also made to identify the potential fungal and bacterial biocontrol agents, particularly *T. harzianum*, *T. virens* and *P. fluorescens* from the farmers' fields growing tomato. Different isolates of potential biocontrol agents i.e. *T. harzianum* (12 numbers), *T. virens* (6 numbers) and *P. fluorescens* (17 numbers) were isolated and identified from rhizosphere soil of healthy plants collected from sick fields from different localities.

Pathogenicity tests conducted in green house on tomato cv. K 25, revealed the pathogenic potential of *M. incognita* and caused significant reduction in the number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight at all initial inoculum levels. In general, there was a positive relationship between the initial inoculum levels of *M. incognita* and reduction in all the test parameters. There was a negative relationship between initial inoculum densities and rate of nematode multiplication. Maximum root-knot index (4.00) and lowest nematode multiplication rate Rf (4.9) were observed at the highest Pi (25,000 J₂/5 kg soil), whereas, highest Rf (15.3) was observed at minimum Pi (2,500 J₂/5 kg soil).

Effect of different inoculum levels of *F. oxysporum* on disease development and on growth and fruit yield of tomato cv. K-25, exhibited a gradual increase in the reduction of number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight of tomato, and these increasing levels also increased percent root infection by the fungus. The maximum reduction in the corresponding test parameters was 39.6, 50.0, 34.7, 36.2,

37.1, 35.5, 40.3 and 38.3%, respectively at the highest initial inoculum level of 1.5×10^7 cfu/5 kg soil as compared to uninoculated control. At the lowest Pi (2.5×10^6 cfu/5 kg soil), the root infection was 4.5% and at the highest Pi (1.5×10^7 cfu/5 kg soil), it was 50.5%.

Different initial inoculum levels of *R. solani* also exhibited its pathogenic potential on tomato cv. K-25 in terms of a gradual increase in the extent of reduction in number of fruits, fruit weight, shoot height, root length, shoot and root fresh and dry weight and also an increase in the percent root infection by the fungus. The maximum reduction in the corresponding test parameters was 53.8, 60.8, 45.2, 40.0, 42.1, 41.4, 45.6 and 45.5%, respectively at the highest initial inoculum level of 15.0 g mycelium/5 kg soil as compared to uninoculated control. At the lowest Pi (2.5 g mycelium/5 kg soil), root infection was 5.0% and at the highest Pi (15 g mycelium/5 kg soil), it was 65.0%.

Studies pertaining to the sequential, simultaneous and single inoculation of *M. incognita* (5000 J₂/5 kg soil), *F. oxysporum* (7.5×10^6 cfu/5 kg soil) and *R. solani* (7.5 g mycelium/5 kg soil) on tomato cv. K-25 indicated that the highest reduction in fruit yield (83.3%), shoot dry weight (78.0%) and root dry weight (81.3%) was found in plants inoculated simultaneously with *M. incognita*, *F. oxysporum* and *R. solani* followed by *M. incognita* seven days prior to *F. oxysporum* and *R. solani*, *F. oxysporum* and *R. solani* seven days prior to *M. incognita*, *M. incognita* and *R. solani* simultaneously, *M. incognita* seven days prior to *R. solani*, *M. incognita* and *F. oxysporum* simultaneously, *M. incognita* seven days prior to *F. oxysporum*, *F. oxysporum* and *R. solani* simultaneously, *R. solani* seven days prior to *M. incognita*, *F. oxysporum* seven days prior to *M. incognita*, *M. incognita* alone, *R. solani* alone and *F. oxysporum* alone, respectively. However, the highest reproduction rate (11.8) of *M. incognita* and root-knot index (2.90) were observed in plants inoculated with the nematode alone, whereas, highest root infection (80.0%) by fungal pathogens was observed in plants inoculated with *M. incognita*, *F. oxysporum* and *R. solani* simultaneously.

In vitro screening of twelve isolates of *T. harzianum*, six isolates of *T. virens* and seventeen isolates of *P. fluorescens* against *F. oxysporum* and *R. solani*, using dual culture method, indicated that isolate TH-H-3 of *T. harzianum*, isolate TV-K-3 of *T. virens* and isolate PS-4 of *P. fluorescens* were the most promising as they significantly ($p \leq 0.05$) inhibited the mycelial growth of both the test pathogens. However, the bioefficacy of

biocontrol agents varied with the pathogens. Similarly, the efficacy of organic amendments (neem seed powder and farmyard manure) and pesticides (carbofuran, topsin-M and bavistin) was evaluated *in vitro* against *F. oxysporum* and *R. solani*. Among all the selected biocontrol agents, organic amendments and pesticides, cent percent inhibition in the mycelial growth of both the pathogens was observed in treatments with bavistin and topsin-M followed by *T. harzianum* isolate TH-H-3 (85.2, 100), *T. virens* isolate TV-K-3 (86.4, 92.2), *P. fluorescens* isolate PS-4 (65.9, 73.3), neem seed powder (21.1, 13.3), carbofuran (11.1, 3.3) and farmyard manure (6.7, 1.1), respectively.

Comparative efficacy of all treatments including promising isolates of biocontrol agents viz., *T. harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 and *P. fluorescens* isolate PS-4 (50 mg/kg soil each), organic amendments viz., neem seed powder (250 mg/kg soil) and farmyard manure (1500 mg/kg soil) and pesticides viz., carbofuran (33.4 mg/kg soil), topsin-M (1.4 mg/kg soil) and bavistin (2 mg/kg soil) was studied in pot conditions against the management of disease complex of *M. incognita* alone, *F. oxysporum* alone, *R. solani* alone, *F. oxysporum* + *R. solani* simultaneously, *M. incognita* + *F. oxysporum* simultaneously, *M. incognita* + *R. solani* simultaneously and *M. incognita* + *F. oxysporum* + *R. solani* simultaneously infesting tomato cv. K-25.

Among the various treatments, carbofuran was found highly effective against *M. incognita* in increasing the plant growth and fruit yield and reducing the root-knot index followed by neem seed powder, *T. harzianum*, *P. fluorescence*, *T. virens*, bavistin, topsin-M and farmyard manure, as compared to untreated inoculated plants.

However, in pots inoculated with *F. oxysporum* alone, *T. harzianum* gave maximum increase in plant growth and fruit yield and reduced the percent root infection. Other effective treatments in descending order were bavistin, *P. fluorescence*, topsin-M, *T. virens*, neem seed powder, carbofuran and farmyard manure.

The highest increase in plant dry weight and fruit yield and lowest root infection by *R. solani*, was also found in plants treated with *T. harzianum* followed by topsin-M, *P. fluorescence*, bavistin, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively.

In plants inoculated with *F. oxysporum* and *R. solani* simultaneously, the treatment with *T. harzianum* was found highly effective in increasing plant dry weight and fruit yield

and in decreasing root infection by both the pathogens followed by bavistin, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, carbofuran and farmyard manure, respectively as compared to untreated inoculated plants.

In simultaneously inoculated plants with *M. incognita* + *F. oxysporum*, *T. harzianum* appeared to be highly effective in increasing plant dry weight and fruit yield and in decreasing root infection by the fungus, followed by carbofuran, *P. fluorescens*, bavistin, *T. virens*, neem seed powder, topsin-M and farmyard manure, respectively as compared to untreated inoculated plants.

Highest plant dry weight and fruit yield and lowest root-knot index and root infection due to combined inoculation of *M. incognita* and *R. solani* was found in plants treated with *T. harzianum* followed by carbofuran, *P. fluorescens*, *T. virens*, topsin-M, neem seed powder, bavistin and farmyard manure, respectively as compared to untreated inoculated plants.

In mixed inocula of *M. incognita*, *F. oxysporum* and *R. solani*, the most satisfactory results were obtained with the treatment of *T. harzianum* followed by *P. fluorescens*, *T. virens*, carbofuran, bavistin, neem seed powder, topsin-M and farmyard manure, respectively. These treatments were found to improve plant growth and fruit yield of tomato cv. K25, thereby decreased root-knot index and percent root infection.

The compatibility test of bioinoculants and there of with organic amendments and pesticides was studied *in-vitro* and the observations indicated that *T. harzianum* isolate TH-H-3 and *T. virens* isolate TV-K-3 were found to be 100 % and 77.8 % compatible, respectively with *P. fluorescens* isolate PS-4. However, in the case of organic additives, all the biocontrol agents were 100 % compatible with neem seed powder and farmyard manure. In case of pesticides, all the biocontrol agents were found to be 100 % compatible with carbofuran. *T. harzianum* and *T. virens* and 100 % incompatible with topsin-M and bavistin. While, *P. fluorescence* was found 100 % compatible with both the fungicides.

The compatible and effective treatment components *viz.*, bioinoculants, pesticides and organic amendments were also evaluated for their efficacy against *M. incognita*, *F. oxysporum*, *R. solani*, using alone and in combinations under pot conditions. Various treatments applied were *T. harzianum* isolate TH-H-3, *T. virens* isolate TV-K-3 and *P. fluorescens* isolate PS-4 @ 100 kg/ha, organic amendments *viz.*, neem seed powder @ 500

kg/ha and farmyard manure @ 3,000 kg/ha and pesticides viz., carbofuran, topsin-M, bavistin @ 2 kg a.i./ha each alone and in combinations [rate of application was reduced to half (for integration of two additives), one third (for integration of three additives) and one fourth (for integration of four additives) of the standard rate] under nursery beds in sick field conditions. Results indicated that all treatments applied singly or in combinations were able to increase the number of emerging seedlings and their fresh weight coupled with considerable reduction in disease incidence. Among all the treatments applied singly, bavistin was found most effective in increasing the emergence of number and fresh weight of seedlings and reducing the disease incidence. When one half dose was applied in the combined treatments of two additives, *P. fluorescens* + bavistin was found the best treatment in increasing the number of emerging seedlings and their fresh weight and in reducing the disease incidence. In case of integrated treatment with three additives applied as one third dose of the standard doses, the treatment with *T. harzianum* + neem seed powder + carbofuran proved best in increasing the number of emerging seedlings and their fresh weight and in reducing disease incidence. Among all integrated treatments with four additives applied as one fourth dose of the standard dose, the *T. harzianum* + farmyard manure + neem seed powder + carbofuran was found highly effective in increasing germination (185.6%) and fresh weight of seedlings (262.7%) and in reducing disease incidence [RKI (0.0) and PRI (7.5)].

The effective treatments or their combinations were then tested under sick field conditions for the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex on tomato cv. K-25. The results indicated that all component of treatments used alone or combined irrespective of doses, considerably increased plant growth and fruit yield and reduced disease intensity as compared to untreated control. *T. harzianum* + farmyard manure + neem seed powder + carbofuran (all the additives applied as one fourth doses of standard dose) was found most effective in increasing plant growth and in reducing disease intensity followed by *P. fluorescens* + farmyard manure + neem seed powder + carbofuran (one fourth doses), *T. harzianum* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + farmyard manure + neem seed powder + bavistin (one fourth doses), *T. harzianum* + neem seed powder + carbofuran (one third doses), *T. virens* + farmyard manure + neem seed powder + carbofuran (one fourth

doses), *P. fluorescens* + farmyard manure + neem seed powder + topsin-M (one fourth doses), *P. fluorescens* + neem seed powder + carbofuran (one third doses), *P. fluorescens* + neem seed powder + bavistin (one third doses), *T. virens* + neem seed powder + carbofuran (one third doses), *T. harzianum* + *P. fluorescens* + neem seed powder (one third doses), *T. virens* + *P. fluorescens* + farmyard manure + neem seed powder (one fourth doses), *P. fluorescens* + neem seed powder + topsin-M (one third doses) and *T. harzianum* + farmyard manure + carbofuran (one third doses), respectively.

The studies have, thus, revealed that biocontrol agents and organic amendments are the most important component package of integrated management system as they have proved effective for reducing the infectivity of *M. incognita*, *F. oxysporum* and *R. solani* disease complex of tomato. In these studies, the treatments have been evaluated under pot, nursery and sick field conditions, wherein high population densities of the pathogens were present. In the farmers' fields, the inoculum levels may vary from low to high, but even in high inoculum densities, the integrated treatments proved a better option for the management of disease complex, and thus, enhancing yield.

The studies have generated knowledge on the damage caused by *M. incognita* - *F. oxysporum* - *R. solani* disease complex of tomato and the development of effective integrated management options. This information is not only of academic importance, but it also has usefulness for tomato growers in increasing the productivity per unit area. It is concluded that farmers should have prior information of their field/soil infestation level with these pathogens and if needed may adopt proper management options to save the crop from this disease complex.

On the basis of the above studies carried out for Ph. D. work, application of *T. harzianum* or *P. fluorescens* (25 kg/ha) + farmyard manure (750 kg/ha) + neem seed powder (125 kg/ha) + carbofuran (16.5 kg/ha) may be recommended to get the best results for the management of *M. incognita*, *F. oxysporum* and *R. solani* disease complex in tomato under large scale cultivation.

CHAPTER-7

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